Sustained-Release Celecoxib from Incubated Acrylic Intraocular Lenses Suppresses Lens Epithelial Cell Growth in an *Ex Vivo* Model of Posterior Capsule Opacity

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Abstract

Purpose: To determine whether celecoxib (CXB) can be released from incubated intraocular lenses (IOLs) sufficiently to inhibit lens epithelial cell (LEC) growth in an *ex vivo* model of posterior capsule opacification (PCO).

Materials: LEC growth was evaluated for 14 days in canine lens capsules (LCs) that had been exposed to media containing 20 μ M CXB for 1–5 days. After the incubation of hydrophilic and hydrophobic IOLs in CXB solution, the determination of the *in vitro* release of CXB from the IOLs was performed for up to 28 days. The incubated and nonincubated IOLs were evaluated in the *ex vivo* model of PCO, and the rate of LEC growth was evaluated over 28 days.

Results: The treatment of LCs with 20 μ M CXB for 4 and 5 days completely inhibited LEC growth. LEC repopulation did not occur after the removal of CXB. IOLs incubated in CXB for 24 h resulted in a sustained release of CXB *in vitro* at levels theoretically sufficient to inhibit PCO. LCs in the *ex vivo* model of PCO treated with acrylic IOLs incubated in CXB had significantly suppressed LEC ingrowth compared with untreated and IOL-only LCs.

Conclusions: A 4-day treatment of LCs with a concentration of 20 μ M CXB may effectively prevent PCO. IOLs incubated in CXB for 24 h resulted in a sustained release of CXB *in vitro* at levels sufficient to inhibit LEC growth in the *ex vivo* model of PCO. Further studies are needed to determine whether CXB-incubated IOLs can effectively prevent the development of PCO *in vivo*.

Introduction

CATARACT SURGERY is one of the most common intraocular surgeries performed worldwide. The removal of the cataract via phacoemulsification and aspiration with intraocular lens (IOL) implantation is the preferred surgical procedure.¹ Although cataract surgery has a high success rate, posterior capsule opacification (PCO) remains the most prevalent long-term complication.^{2–4} After cataract surgery, residual lens epithelial cells (LECs) undergo an epithelial-mesenchymal transition (EMT), resulting in LEC proliferation and migration across the posterior lens capsule (LC).^{5–7} This process results in a fibrosis-type PCO that causes secondary vision loss.^{2,4} Advances in IOL design, IOL materials, and improvement of surgical techniques have, to some extent, reduced the incidence and severity of PCO.^{2,8,9} However, decreased visual acuity from PCO occurs in up to 50% of adults, and the rate of PCO in children ranges from 43.7% to

100% after cataract surgery.⁴ PCO can be effectively treated by neodymium:yttrium-aluminum-garnet (Nd:YAG) laser capsulotomy, but this procedure is associated with a high cost and risk of significant complications, such as retinal detachment, damage to the IOL, increase in intraocular pressure, and IOL subluxation.^{3,4} Therefore, the development of alternative and improved methods that prevent PCO is needed.

The pathogenesis of PCO has been investigated, and pharmacologic prevention has been attempted using various substances *in vitro*. Growth factors, such as transforming growth factor beta and fibroblast growth factor, have been implicated in PCO formation by promoting proliferation of LECs and cell matrix deposition.^{10–12} In addition, specific pathways, such as intracellular calcium signaling, are associated with PCO development.¹³ However, the effect of inhibition of specific components of the signaling pathway on PCO prevention was found to be unsatisfactory or showed inconsistent results,^{2,14} which suggests that PCO develops

through multiple redundant pathways involving various factors.

Pharmacological methods using 5-fluorouracil, mitomycin C, lidocaine, carbachol, and osmotic agents have been shown to be effective in the inhibition of PCO *in vitro*.^{2,15} However, some of these drugs have a toxic effect on the surrounding intraocular tissues, especially on the corneal endothelium, thus limiting their clinical use. Devices that allow isolated lens capsular exposure to drugs, such as the Perfect Capsule device (Milvella, Ltd., Epping, Australia), thus limiting toxicity to the remainder of the eye, are promising, but further development is needed before clinical use.¹⁶⁻¹⁸

IOL design, IOL materials, and surface modifications have been studied in an attempt to prevent PCO with variable degrees of success.^{2,8,9,19-22} The use of the IOL itself, or an attached drug delivery device, has been recently considered as delivering drugs to decrease PCO, postoperative inflammation, or prevent infectious endophthalmitis.²³⁻²⁹ Possibly, the most effective way to deliver drugs to the LC after cataract surgery would be via the IOL material itself because of the large area of the capsule-IOL contact and the adjacent location of the IOL to the site of action (ie, the LECs on the LC). Recent studies have demonstrated success in the delivery of drugs to the eye after incubating or coating IOLs in antibiotics,^{25,28,30} indomethacin,³¹ dexamethasone,²⁴ or rapamycin.³² For the IOL-drug incubation/sustained release to be effective in the prevention of PCO, one should determine whether sufficient concentrations of the drug in question can be released for appropriate durations to inhibit LEC growth, and, thus, PCO, without causing toxicity to the eye.²

Recently, it has been demonstrated that cyclooxygenase-2 (COX-2) expression was elevated in the LECs of canine cataract patients and in an *ex vivo* lens model of PCO.^{33,34} Furthermore, incubation with 10 and 20 μ M celecoxib (CXB), a specific COX-2 inhibitor, in an *ex vivo* LC PCO model induced apoptosis in LECs and resulted in the inhibition of cell proliferation. This inhibition of the LEC's growth was shown to be a pharmacological effect rather than nonspecific toxicity.³⁴ Inhibition of the LEC's growth occurred after incubation with CXB for 7 days, and LEC growth did not resume when these cultures were continued without CXB for another 7 days.³⁴

The purpose of the studies in this article was to determine whether CXB can be released from IOLs sufficiently to inhibit LEC growth in an *ex vivo* model of PCO. These studies involved determining the minimal exposure time of LECs to CXB that is required to prevent LEC growth and measuring the *in vitro* release of CXB from incubated acrylic IOLs. Finally, use of the CXB-incubated IOLs in an *ex vivo* model of PCO was done to determine the effectiveness of this drug delivery method on LEC growth and, thus, on development of PCO.

Methods

Duration of CXB required to inhibit PCO

The purpose of these experiments was to determine the minimum period of exposure of LECs to CXB that is required to inhibit LEC growth in an *ex vivo* model of PCO. Normal eyes were collected from dogs from a local county animal shelter, and they were processed within 3 h of euthanasia. All the dogs used in this study were in good health and esti-

mated to be between 1 and 5 years of age. The use of animals in this study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was reviewed and monitored by the North Carolina State University Institutional Animal Care and Use Committee.

Ex vivo LC model of PCO

The *ex vivo* model of PCO was used as previously described.³⁴⁻³⁶ A routine continuous curvilinear 5 mm-diameter capsulorhexis was performed. The lens nucleus and cortex were removed intact through the capsulotomy site, and residual cortex from the LC was removed by irrigation with balanced salt solution (BSS). Lenticular zonules were severed, and the LC was gently excised from its vitreal attachments. Each LC was then placed in a sterile Petri dish with the capsulotomy side up and pinned into place using evenly spaced sterilized entomology pins. Five milliliters of 20 μ M CXB in Dulbecco's Modified Eagle's Medium (DMEM) containing antibiotics and antimycotics (Mediatech, Manassas, VA) were added to the immobilized LC. The cultures were maintained at 37°C with 5% CO₂.³⁴⁻³⁶ The LC were exposed to 20 μ M CXB for 1, 2, 3, 4, or 5 days. After the exposure time, the media containing CXB was removed and replaced with DMEM, then replaced routinely every 4 days thereafter for a total of 14 days. Experiments were performed in triplicate. As a control, 1 LC exposed to DMEM only was added to each exposure time group with identical media exchange. LEC growth on capsules was monitored and photographed every 24 h using an inverted microscope, and the percentage of total cell area that covered the LC was recorded. After 14 days of culture, LCs were removed from the Petri dish, fixed in 4% paraformaldehyde, and processed for routine histopathology with hematoxylin and eosin staining. The degree of LEC growth, adherence to LCs, and cellular vacuolation was graded 0-4. (0=none, 1=1%-25%, 2=26%-50%, 3=51%-75%, and 4=76%-100%).³⁴

Sustained release of CXB from acrylic IOLs

Hydrophobic (Acrysof; Alcon, Fort Worth, TX) or hydrophilic (Acri.Tec; Zeiss, Berlin, Germany) acrylic IOLs were placed in 5 mL of 100 μ M CXB solution [1.0% dimethyl sulfoxide (DMSO)] or 1 $\times$ phosphate-buffered saline (PBS) for 1, 8, or 24 h, in triplicate. The IOLs were transferred to individual vials containing 1 mL of 1 $\times$ PBS (pH 7.4) and maintained in a water bath at 37°C. The IOLs were removed, blotted dry, and placed into new vials with 1 mL of 1 $\times$ PBS every 24 h for 7 consecutive days. CXB concentrations in the PBS were determined using a reversed-phase high-performance liquid chromatography (HPLC) assay and reported as μ g/mL/day.

Since canine LCs were used in the *ex vivo* model of PCO to determine whether delivery of CXB from IOLs could inhibit LEC growth, the IOL experiments just mentioned were repeated, with some experimental modifications, using specific canine-designed hydrophilic acrylic IOLs (60V Acrivet; Zeiss). One modification was that these IOLs were placed in a higher concentration of CXB (5 mL of a 300 μ M CXB solution in 25% DMSO) or 1 $\times$ PBS with 25% DMSO for 24 h, in triplicate. The higher concentration of incubation solution was used in an attempt to maximize the CXB drug load in the IOL and, thus, resulted in higher release rates for the

larger canine LC. After incubation, the canine IOLs were transferred to individual vials containing 1 mL of 1×PBS (pH 7.4) and maintained in a water bath at 37°C. The IOLs were removed, blotted dry, and placed into new vials with 1 mL 1×PBS every 24 h. A second experimental modification compared with the human IOLs was that the *in vitro* release rates of the canine IOLs were measured for 28 days after incubation. CXB concentrations in the PBS were determined using a reversed-phase HPLC assay and reported as µg of drug release/mL/day.

Drug analysis

CXB concentrations in the PBS were determined using HPLC with ultraviolet detection at 254 nm (Alliance™ 2795 Separations Module; Waters Corporation, Milford, MA). Separation was achieved on a C18 column maintained at 40°C. The mobile phase consisted of deionized and filtered water and HPLC grade acetonitrile (42:58 v/v) run at a flow rate of 1 mL/min. The samples were injected directly onto the machine without extraction at an injection volume of 50 µL. Concentrations of each drug were determined from a calibration curve in the mobile phase made before each run. Calibration curves were considered linear over a range of 0.01–10 µg/mL for all drugs if the coefficient of determination (R^2) was greater than 0.99 and the calculated values for each concentration were within 15% of the true value.

Lens epithelial growth after placement of CXB-incubated IOLs

Canine acrylic IOLs (60V; Acrivet, Hennigsdorf, Germany) were incubated for 24 h in 1×PBS/25% DMSO solution only or a 300 µM solution of CXB (in PBS/25% DMSO). Incubated and nonincubated IOLs were rinsed with 1 mL of BSS, and placed into the LC of the *ex vivo* PCO model that was created using cadaver eyes from young normal dogs, as just described.^{34–36} The size of the capsulotomy opening (~5 mm diameter) was 1 mm less than the IOL optic diameter. The IOLs were placed into the LC without the aid of viscoelastics or any other material except for BSS. Additionally, LCs without any treatment were set up at the same time as the IOL studies. Each LC was then placed in a sterile Petri dish with the capsulotomy side up. Five milliliters of DMEM containing antibiotics and antimycotics (Mediatech) was added to the LCs. The cultures were maintained at 37°C with 5% CO₂.^{34–36} The DMEM was replaced routinely every 4 days to maintain the cultures and to partially mimic aqueous humor turnover. The cultures were maintained for a total of 28 days. LEC growth on LCs was monitored and photographed every 24 h for 5 days using an inverted microscope, then at days 7–11, 14, 17, 21, 25, and 28. The percentage of the total area of cellular ingrowth on the posterior LC beneath the IOL, or the posterior LC below the 5 mm anterior capsulotomy in LCs without IOLs, was estimated and recorded. Photographs were used to determine the percent cellular ingrowth via image analysis software (ImageJ, v 1.43.). After 28 days of culture, the LCs were removed from the media, fixed in 10% formalin, and processed for routine H&E staining. The degree of LEC growth, adherence to LCs, and cellular vacuolation was graded 0–4. (0=none, 1=1%–25%, 2=26%–50%, 3=51%–75%, and 4=76%–100%).³⁴

Statistical and data analysis

Pharmacokinetic analysis of drug concentrations was performed using specialized commercially available software (WinNonLin 4.0.1; Pharsight Corporation, Mountain View, CA). Noncompartmental analysis was used to determine the elimination half-life ($t_{1/2}$) of the drug from the IOLs during the sampling period, using the equation $t_{1/2}=0.693/\lambda_z$, where λ_z is the slope of the terminal portion of the elimination curve. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was also calculated using the trapezoidal rule, as a marker for total drug exposure. Differences in CXB release rates, $t_{1/2}$ and $AUC_{0-\infty}$, within each incubation time and within each day for each type of IOL were analyzed using repeated-measures analysis of variance with Tukey's test. Differences in CXB release rates, $t_{1/2}$ and $AUC_{0-\infty}$, between the IOLs were analyzed using a paired *t*-test.

Differences in the degrees of cellular ingrowth of LEC and adherent cells on *ex vivo* LCs were analyzed by the Wilcoxon rank-sum test. Statistical analysis of the data was performed using computerized software (JMP® Statistical Discovery Software; SAS Corporation, Cary, NC). Significance was set at $P \leq 0.05$ for all comparisons.

Results

Duration of CXB required to inhibit PCO

On examination of canine LCs after 1 day of culture, LECs were present in all groups and visible in 10%–30% of the entire LC area, most predominantly in the peripheral LC. The LECs on the LCs exposed only to DMEM demonstrated proliferation occurring within 48 h of culture. Cell proliferation began near the equator of the capsules and continued toward the center on the anterior and posterior LCs for up to 14 days of culture. In addition, LECs developed changes consistent with EMT beginning from day 3 to 5 days of culture and continuing through day 14. The LECs that had been treated with 20 µM CXB for 1, 2, or 3 days demonstrated the same pattern and rate of cellular growth and EMT as the control capsules. However, the LECs treated with CXB for 4 and 5 days did not proliferate beyond the initial 10%–30% confluency, and no changes were observed throughout the 14 days of culture after removal of the CXB. The cellular growth rate (% of total capsule area) in 4- and 5-day CXB-treated capsules did not increase throughout the 14 days, in contrast to the other LCs (Fig. 1). There were no significant differences in the mean LEC growth rate among all the groups on day 1 or between 4- and 5-day CXB-treated capsules at any time. By day 5, a significant decrease in cellular growth was observed in the LCs that had been treated with CXB for 5 days compared with those which had been treated with CXB for 1 day or all non-CXB treated capsules ($P < 0.05$). By day 7, cellular growth in the 4- and 5-day treatment groups was significantly less than that in the 1- and 3-day CXB treatment groups or non-CXB treated capsules ($P < 0.05$). At days 11 and 14, cellular growth in the 4- and 5 day treatment groups was significantly less than in the 1-, 2-, and 3 day CXB treatment groups and non-CXB treated LCs ($P < 0.05$) (Fig. 1).

Histopathology revealed significantly fewer cells on the LCs treated with CXB for 4 and 5 days than the control (day 4: $P = 0.0236$; day 5: $P = 0.0211$) (Figs. 2 and 3). Furthermore, the degree of adhesion of LEC to LCs treated with CXB for 4

FIG. 1. Cellular growth rate (% of total cell area) of lens epithelial cells (LECs) in *ex vivo* posterior capsule opacification (PCO) models over 14 days duration. a, cellular growth in Group 5 is significantly less than Group 1 ($P < 0.05$); b, cellular growth in Group 4 is significantly less than Group 1 ($P < 0.05$); c, cellular growth in Group 5 is significantly less than Group 3 ($P < 0.05$); d, cellular growth in Group 4 is significantly less than Group 3 ($P < 0.05$). Control denotes all LCs exposed to DMEM only. Group 1, 1 day exposure; Group 2, 2 day exposure; Group 3, 3 day exposure; Group 4, 4 day exposure; Group 5, 5 day exposure.

and 5 days was significantly less than the control (day 4: $P = 0.0211$; day 5: $P = 0.0347$). The degree of cellular vacuolation in LCs treated with CXB for 1 day was significantly higher than the control ($P = 0.049$) (Fig. 2); however, evaluation was not possible in LCs treated with CXB for 4 and 5 days due to a very low numbers of cells. Otherwise, there were no differences in histopathology between LCs treated with CXB for 1–3 days and untreated controls.

Sustained release of CXB from acrylic IOLs

CXB was not released in quantifiable amounts from the control IOLs that were incubated in PBS at any time. Mean

FIG. 2. Mean score of cell growth, adherent LEC cells on lens capsules (LCs), and vacuolation in *ex vivo* LC models, by histologic evaluation. a, Group 4 is significantly different from the control ($P < 0.05$); b, Group 5 is significantly different from the control ($P < 0.05$); c, Group 1 is significantly different from the control ($P < 0.05$). 0 = none, 1 = 1%–25%, 2 = 26%–50%, 3 = 51%–75%, and 4 = 76%–100%. Control denotes all LCs exposed to DMEM only. Group 1, 1 day exposure; Group 2, 2 day exposure; Group 3, 3 day exposure; Group 4, 4 day exposure; Group 5, 5 day exposure.

FIG. 3. Histologic sections of canine LCs after 14 days of culture. LC incubated in celecoxib (CXB) during the first 4 days (A) shows complete inhibition of LEC proliferation compared with the control (B).

release of CXB from human IOLs incubated in the CXB solution ranged from $1.38 \mu\text{g/day}$ after 24 h to $0.03 \mu\text{g/day}$ after 7 days of release (Fig. 4). Longer CXB incubation times resulted in a higher release of CXB per day in both hydrophobic and hydrophilic IOLs (Fig. 4). In the case of the hydrophobic IOLs, no significant differences were observed in the release rates on any day when comparing the IOLs incubated for 1 or 24 h. However, for the 8-h incubation period, the release rate on day 1 was significantly higher than that on day 5 ($P = 0.047$). A similar comparison of the hydrophilic IOLs revealed a significantly higher release rate on day 1 compared with that on day 7 for the 1-h incubation period ($P = 0.04$); however, there were no significant differences in the release rates of IOLs incubated for 8 and 24 h. There were no significant differences in release rates when comparing the hydrophobic and hydrophilic IOLs at any time point (Fig. 4).

In the case of the hydrophobic IOLs, there were no significant differences in $t_{1/2}$ of drug release as a result of any of the CXB incubation times. The $\text{AUC}_{0-\infty}$ was significantly higher for the hydrophobic IOLs incubated for 24 h [$151.8 \pm \text{standard deviation (SD)} 26.5 \text{ h} \cdot \mu\text{g/mL}$] compared with the hydrophobic IOLs incubated for 1 h ($37.7 \pm \text{SD } 1.2 \text{ h} \cdot \mu\text{g/mL}$) ($P < 0.0001$). With regard to the hydrophilic IOLs, there were no significant differences in $t_{1/2}$ or $\text{AUC}_{0-\infty}$ between any of the incubation times (Fig. 5). A comparison of the calculated $t_{1/2}$ between the hydrophobic and hydrophilic IOLs revealed no significant differences between the 1

FIG. 4. Mean $\pm$ standard deviation (SD) CXB concentrations per day for hydrophobic (A) and hydrophilic (B) IOLs. a, 8 h incubation significantly higher at 1 day than 5 days ($P=0.047$). b, 1 h incubation significantly higher at 1 day than 7 days ($P=0.04$).

and 8 h incubation times. The $t_{1/2}$ of drug elimination from the hydrophilic IOLs ($98.1 \pm \text{SD } 21.7 \text{ h}$) was significantly longer than that from the hydrophobic IOLs ($62.7 \pm \text{SD } 13.9 \text{ h}$) for the 24-h ($P=0.044$) incubation times. A comparison of the calculated $\text{AUC}_{0-\infty}$ between the hydrophobic and hydrophilic IOLs revealed no significant differences between the 8 and 24-h incubation lenses. However, the $\text{AUC}_{0-\infty}$ from the hydrophilic IOLs was significantly higher than that from the hydrophobic IOLs for the 1-h ($P<0.0001$) incubation time (Fig. 5).

The mean cumulative release of CXB from hydrophilic and hydrophobic IOLs over 7 days in the IOLs incubated in

FIG. 5. Mean $\pm$ SD area under the curve ($\text{AUC}_{0-\infty}$) calculated for hydrophobic and hydrophilic intraocular lenses (IOLs) calculated through the entire period of release (7 days). a, $\text{AUC}_{0-\infty}$ from the hydrophilic IOLs was significantly higher than from the hydrophobic IOLs for the 1 h ($P<0.0001$) incubation time; b, $\text{AUC}_{0-\infty}$ was significantly higher for the hydrophobic IOLs incubated for 24 h compared with the hydrophobic IOLs incubated for 1 h ($P<0.0001$).

FIG. 6. Mean $\pm$ SD CXB concentration (μg) released per day in phosphate-buffered saline from canine hydrophilic IOLs that had been incubated in $300 \mu\text{M}$ CXB solution for 24 h. a, release significantly higher than days 5 through 28 ($P<0.0001$); b, release significantly higher than days 14 through 28 ($P=0.0025-0.0296$).

CXB for 24 h was $4.4 \pm \text{SD } 1.1 \mu\text{g}$ and $3.6 \pm \text{SD } 1.4 \mu\text{g}$, respectively.

In the case of the canine IOLs, CXB was not released in quantifiable amounts from the control lenses incubated in PBS at any time. The mean release of CXB from the IOLs incubated in the CXB solution ranged from $1.54 \mu\text{g/day}$ after 24 h to $0.38 \mu\text{g/day}$ after 28 days of release (Fig. 6). CXB release at days 1 and 2 was significantly higher than that at days 5 through 28 ($P<0.0001$). Furthermore, release at day 7 was significantly higher than that at days 14 through 28 ($P=0.0025-0.0296$). No significant differences were found in the release rates from days 8 through 28 (Fig. 6).

With regard to the CXB release from incubated canine hydrophilic IOLs, mean $t_{1/2}$ was calculated to be $542.7 \pm 30 \text{ h}$, and the $\text{AUC}_{0-\infty}$ was $679 \pm 29.2 \text{ h} \cdot \mu\text{g/mL}$. The mean cumulative release of CXB from canine hydrophilic IOLs over 28 days was $10.76 \pm \text{SD } 0.87 \mu\text{g}$.

Lens epithelial growth after placement of CXB-incubated IOLs

LCs without IOLs expressed a cell growth similar to the LCs described earlier, in which cells were proliferating near the equator and growing centrally until reaching confluency by 14 days. LCs with IOLs had LEC proliferation at the optic edge initially, followed by growth along the LC anterior and posterior to the IOL. LCs with IOLs incubated with either a vehicle or CXB had significantly less LEC growth on days 1 through 11 of culture compared with the untreated LCs or those treated with a nonincubated IOL (Fig. 7). The LCs treated with nonincubated IOLs had significantly less LEC growth than the untreated LCs on days 7–11 of culture. The LCs treated with CXB-incubated IOLs had significantly less LEC growth than the LCs without IOLs, those with non-incubated IOLs, and those with vehicle-incubated IOLs on days 11–28. Furthermore, all LCs, except those with IOLs treated with CXB, had LC folds indicative of EMT (Fig. 8). LCs without treatment attained LEC confluency by 14 days, LCs with untreated IOLs and vehicle-incubated IOLs attained $>95\%$ confluency by 25 days, while CXB-incubated IOLs reached less than 90% confluency by 28 days of culture (Figs. 7 and 8).

FIG. 7. Mean $\pm$ SEM percentage of confluency of lens epithelium over 28 days of culture in the *ex vivo* canine PCO model. LCs (LCs) had placement of IOLs that were CXB-incubated (60V + CXB), vehicle-incubated (60V + vehicle), or nonincubated (60V IOL only). Additionally, the percentage of confluency of lens epithelial growth was evaluated in untreated LC (LC only). a, 60V + Vehicle and 60V + CXB significantly less than LC only and 60V IOL only (Day 2: $P < 0.006$); (Day 3: $P = 0.03$; $P = 0.021$); (Day 4: $P = 0.02$; $P = 0.0006$). b, 60V + Vehicle and 60V + CXB significantly less than LC only and 60V IOL only (Day 7: $P < 0.0001$); (Day 8: $P < 0.0001$); (Day 9: $P < 0.0001$); (Day 10: $P < 0.0001$). c, 60V IOL only significantly less than LC only (Day 7: $P = 0.0008$); (Day 8: $P < 0.0001$); (Day 9: $P = 0.0001$); (Day 10: $P = 0.0013$). d, 60V + CXB, 60V + Vehicle, 60V IOL, and LC only all significantly different ($P < 0.0001$). e, 60V + Vehicle and 60V IOL significantly less than LC only ($P < 0.0007$). f, 60V + CXB significantly less than 60V + Vehicle, 60V + CXB, and LC only ($P < 0.0234$). g, 60V + Vehicle significantly less than 60V IOL and LC only ($P = 0.005$).

FIG. 8. Inverted microscopic images of the *ex vivo* canine LC model of PCO. IOLs incubated in CXB (D) exhibited significantly less LEC growth than IOLs incubated in a vehicle (C), those with unincubated IOLs (B), and LCs without IOLs (A) from days 11 to 28 of culture. Arrows show LC folds indicative of epithelial-mesenchymal transition-like changes. (A) LC without IOLs at 15 days of culture; (B) LC with unincubated 60V IOLs at 28 days of culture; (C) LC with 60V IOLs incubated in a vehicle (with 25% dimethyl sulfoxide); (D) LC with CXB-incubated 60V IOLs. Magnification is 20 $\times$.

Histologically, the percentage of cellular growth in the LCs treated with 60V IOLs incubated in CXB was significantly less than in the untreated LCs, LCs treated with the canine IOLs alone, or those with IOLs incubated with a vehicle ($P = 0.012$) (Fig. 9). There were no significant differences in the mean histologic score of the percentage of attachment of LECs on LCs or vacuolation of cells (Fig. 9).

Discussion

Results of this study demonstrate that incubation of *ex vivo* canine LCs in 20 μ M CXB for a minimum of 4 days completely inhibited the growth of LEC, and growth of residual peripheral LECs did not occur after removal of the CXB. EMT is a change in the phenotype of LECs from an epithelial to a fibrous metaplastic morphology accompanied by aberrant basement membrane synthesis.^{5,6} It has been shown that LECs undergoing EMT over-express COX-2, α -smooth muscle actin (α -SMA), lumican, the transcriptional repressors Slug and Snail, telomerase reverse transcriptase (TERT) and telomerase, and phosphorylated Akt, a survival factor.^{6,37–39} CXB is a highly selective, nonsteroidal anti-inflammatory COX-2 inhibitor.⁴⁰ CXB also inhibits Akt and proliferation of cells by down-regulation of cyclins, resulting in cell-cycle arrest. It has a pro-apoptotic effect mediated by caspase-3 activation.^{41,42} In canine LCs treated with 20 μ M CXB for 2 weeks, the LECs showed decreased expression of COX-2, inhibition of proliferation cell nuclear antigen and α -SMA, and up-regulation of caspase-3.³⁴ Therefore, these results suggest that incubation of the *ex vivo* LC cultures with CXB effectively inhibited EMT of LECs by decreasing migration and proliferation and inducing apoptosis through caspase-3 activation.³⁴ It was also demonstrated that these effects

FIG. 9. Mean histologic score of cell growth, attachment of LEC cells on LCs, and vacuolation of cells in an *ex vivo* PCO model treated with canine IOLs (60V IOL) alone, with a vehicle, or after incubation in CXB. The percentage of cellular growth in the LC treated with 60V IOL incubated in CXB (a) was significantly less than that obtained with the other treatments ($P=0.012$). Histologic score: 0 = none, 1 = 1%–25%, 2 = 26%–50%, 3 = 51%–75%, and 4 = 76%–100%.

were due to pharmacological activity rather than to cellular toxicity.

Earlier, incubation of a 20 μM concentration of CXB for 7 days completely inhibited LEC growth in an *ex vivo* model of PCO,³⁴ while our results, using the same model of PCO, indicate that incubation with 20 μM CXB for a minimum of 4 days was required to completely inhibit the growth of LECs. We also found that adhesion of LECs to LCs was less in capsules treated for 4 and 5 days, compared with vehicle control or treatment groups of 3 days or less. Therefore, 4 days is the target duration for delivery of CXB to the LC to potentially prevent PCO. Moreover, COX-2 and its metabolic products such as prostaglandin E2 (PGE2) induce inflammation and are major inflammatory mediators in uveitis. Sustained release of CXB, therefore, would act to suppress lens-induced uveitis, while, at the same time, directly inhibiting the development of PCO after cataract surgery.

Sustained release of CXB was demonstrated from several types of acrylic IOLs in this study. Selection of the 1 mL volume of PBS to perform the *in vitro* release of CXB from IOLs was selected to better approximate the LC and aqueous humor volume in which the IOLs would release CXB *in vivo*. However, although CXB is weakly acidic and has a very low saturated solubility in water at 3–7 $\mu\text{g/mL}$ at pH 7.0 and 40°C,⁴³ “sink” conditions were not likely achieved using the 1 mL of PBS. The highest daily release of CXB (approximately 1.5 $\mu\text{g/mL}$) was measured during the first 24 h of release, which resulted in a concentration of ~20%–46% of CXB saturation (not a concentration of less than 1/3 of saturation required for “sink” conditions).⁴⁴ However, the release of CXB after 24 h was ~1 $\mu\text{g/mL}$, and, therefore, “sink” conditions were likely maintained for the remainder of the release rate period. Therefore, the lack of achieving “sink” conditions over the first 24 h of CXB release likely had little effect on the overall release kinetics. Ideal perfect sink conditions would require that the drug be tested in fresh solvent, where there is no build up of dissolved drug in the dissolution medium. This would require a flow-

through type apparatus,⁴⁵ which was not available for this study.

Human hydrophilic IOLs had significantly longer CXB $t_{1/2}$ (24 h incubation) and significantly higher $\text{AUC}_{0-\infty}$ (1 h incubation) compared with hydrophobic IOLs and, therefore, exhibited slightly more desirable CXB sustained release pharmacokinetics. Hydrophilic IOLs also had a higher cumulative release over 7 days [$4.4 \pm \text{SD } 1.1 \mu\text{g}$ (24 h incubation)] compared with hydrophobic IOLs [$3.6 \pm \text{SD } 1.4 \mu\text{g}$ (24 h incubation)]. The higher CXB cumulative release and better pharmacokinetic parameters for hydrophilic IOLs are possibly a result of the higher amounts of drug that were loaded into the hydrophilic acrylics during incubation or simply that more of the incubation fluid was able to perfuse into the hydrophilic polymer of the IOL. Differences in IOL design (ie, haptic design; optic size) may have also contributed to the differences in release and drug content in IOLs after incubation, but since these differences are small, the effect on drug delivery is not expected to be high. Therefore, due to the favorable PK parameters observed with the 24 h incubation in human IOLs, this incubation time was used for the canine IOLs to allow the optimal release of CXB for use in the *ex vivo* PCO model.

The canine IOL is slightly larger in volume, has more acrylic polymer than human IOLs, and was incubated in a higher concentration of CXB; therefore, it was expected to be able to deliver higher concentrations of CXB *in vitro*. A higher release rate was thought to be needed because of the larger volume of the canine lens (~300 μL) compared with the human lens volume (160 μL).^{46,47} The CXB release rate, total IOL drug load, and PK parameters were higher in canine IOLs compared with human IOLs, as expected. The highest release of 1.54 $\mu\text{g/mL}$ CXB from the canine IOLs into PBS represents less than ~22%–51% of the maximal solubility of CXB in PBS. What was not expected was the duration of CXB release: At 28 days, release rates were nearly 0.4 $\mu\text{g/day}$. As suggested earlier, the duration of CXB release needs to be short (ie, 4 days) to prevent PCO, and this long release rate may not be required to prevent PCO. However, the sustained release of a low amount of CXB may have other favorable effects, such as reduction of inflammation after cataract surgery. Although the release rates were higher with the canine IOL (1.54 $\mu\text{g/mL/day}$ at day 1; cumulative release of 6.0 μg by day 7) compared with the human IOL (1.38 $\mu\text{g/mL/day}$ at day 1; cumulative release of 4.4 μg by day 7), the difference was small (difference of <0.16 μg CXB released/day at day 1; difference <1.6 μg cumulative release by day 7), which suggests that there is a limit to the amount of drug that can be loaded into the IOL and that higher concentrations of CXB (and the required higher concentrations of DMSO to provide solubility of CXB) may not be required for higher drug delivery.

For the IOL to be an effective drug delivery method, the amount of CXB that can be released from a CXB-incubated IOL should be determined to be sufficient to achieve concentrations in the LC to inhibit the development of PCO. Based on previous studies, concentrations as low as 10 μM of CXB inhibited LEC growth,³⁴ although the minimal concentration required to inhibit LECs has not yet been determined. With volume of a human LC being ~160 μL ,^{46,47} based on *in vitro* release rates of CXB, the concentrations of CXB that can be achieved from human hydrophilic IOLs incubated for 24 h is calculated to be 22.6 μM at 1 day to

9.0 μM at 4 days. The mean volume of the canine lens is $\sim 300 \mu\text{L}$ and based on an *in vitro* CXB release from incubated IOLs; the concentrations of CXB in canine LCs that can be achieved is calculated to be 13.5 μM at 1 day, 6.3 μM at 4 days, and, interestingly, 3.3 μM at 28 days. Therefore, release rates of CXB from incubated IOLs are theoretically within the concentrations of CXB that are known to inhibit LEC growth *in vitro* (10–20 μM) for most of the 4 days required to prevent PCO. These calculations, however, do not take into account that the LC is not a closed system *in vivo* and that released CXB likely escapes the LC into the anterior chamber.

Use of CXB-incubated IOLs in an *ex vivo* canine model of PC resulted in a significant decrease in LEC growth, especially after 11 days of culture. Although there was significant suppression of LEC growth, there was no complete inhibition as was observed with 20 μM CXB in the lens culture media. The lack of complete LEC inhibition with the use of CXB-incubated IOLs in this *in vitro* model of PCO may be a result of a less-than-adequate concentration of CXB released into the relatively large volume (5 mL) of media used in the *in vitro* culture method or because the IOL provided the protection of stressed cells (ie, less agitation), thereby keeping them in the LC. However, LCs treated with IOL alone or a vehicle-incubated IOL had decreased LEC ingrowth, but those incubated with CXB had significantly less ingrowth. The use of IOLs has been demonstrated earlier as decreasing PCO, and this is likely due to the mechanical inhibition of LEC migration.² The DMSO that was in the incubation fluid could have been released into the LC and may have also had some effect on LEC growth; however, this likely had a minimal effect, as many LEC culture protocols use a low concentration of DMSO in the media.⁴⁸ Since significantly decreased LEC growth was observed in the LCs with CXB-incubated IOLs, further studies are warranted to determine whether the effect of this method of CXB delivery is sufficient to inhibit PCO *in vivo*.

Other IOL modifications have been recently evaluated for their effect on PCO.^{2,19,20,23,24,29} A selenocystamine-coated IOL significantly reduced PCO in the canine *ex vivo* LC model without toxicity.²⁰ There was a significant decrease of PCO at the central posterior capsule where IOLs and LCs made contact. However, there was a large amount of cell proliferation at the periphery. It appeared that strong covalent binding inhibited the release of selenocystamine into the lens capsular bag.²⁰ The release of CXB from incubated IOLs, as just discussed, should allow distribution to the entire lens capsular bag and, therefore, inhibit PCO at both the center and periphery of the LC. Finally, the Perfect Capsule device (Milvella, Ltd.) allows the isolated LC exposure to drugs, without leakage of the drug into the anterior chamber, thus limiting toxicity to structures such as uveal tissue or corneal endothelium.^{16–18} The Perfect Capsule device has much promise for the 1-time treatment of the LC with substances such as 5-FU; however, its use would not allow the sustained delivery of drugs, such as CXB. Without the sustained delivery of CXB, additional benefits of the drug on the eye, such as its anti-inflammatory effect, would not likely be observed.

The use of IOLs as drug delivery devices, as described in our studies, has been previously described in an attempt to prevent postoperative infectious endophthalmitis or decrease PCO.^{24,25,27–31} Pharmacokinetic data are not available for all

of these studies; however, in 1 study, IOLs coated with indomethacin had drug release for at least 3 days *in vitro*,³¹ and IOLs coated with rapamycin had drug concentrations detectable for up to 14 days in the aqueous humor of rabbit eyes.³² Both studies demonstrated decreased PCO with treated IOLs compared with control eyes. In another study, the sustained release of 3 matrix metalloproteinase inhibitors from silicone rubber IOL prototypes allowed the sustained release of the drugs for more than 5 months.²³ Therefore, our findings on the sustained release of CXB from the incubated IOL are consistent with previous studies using other drugs.

In summary, incubation of *ex vivo* LCs in 20 μM CXB for 4 and 5 days completely inhibited the growth of LECs, and these findings suggest that a 4-day treatment of LCs with a concentration of 20 μM CXB may effectively prevent PCO. IOLs incubated in CXB for 24 h resulted in a sustained release of CXB *in vitro* at levels theoretically sufficient to inhibit PCO. Furthermore, LCs treated with acrylic IOLs incubated in CXB had suppressed LEC ingrowth compared with untreated and IOL-only LCs. Further *in vivo* studies are needed to determine whether CXB-treated IOLs can effectively prevent the development of PCO.

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Author Disclosure Statement

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