Reduction of Vitreous Prostaglandin E₂ Levels After Topical Administration of Ketorolac 0.45%

Scott D. Schoenberger, MD; Stephen J. Kim, MD; Jinsong Sheng, MD; M. Wade Calcutt, PhD

IMPORANCE Inhibition of proinflammatory prostaglandins in the retina may have therapeutic effects for retinal disease.

OBJECTIVE To determine vitreous levels of ketorolac and prostaglandin E₂ (PGE₂) in eyes treated with topical ketorolac tromethamine 0.45% (Acuvail).

DESIGN, SETTING, AND PARTICIPANTS A prospective comparative interventional study, performed in a university academic hospital, included 24 eyes in 22 consecutive patients undergoing pars plana vitrectomy.

INTERVENTION Application of topical ketorolac 0.45%, 4 times daily, for 3 days before pars plana vitrectomy in the first 12 consecutive eyes. The next 12 eyes were untreated and served as controls. Undiluted vitreous samples were obtained at the time of surgery and immediately frozen at −80°C.

MAIN OUTCOMES AND MEASURES Vitreous ketorolac and PGE₂ levels.

RESULTS Seven of the 12 eyes (58%) had ketorolac levels above the lower limit of quantitation. All 7 were in pseudophakic eyes, and 4 of the 5 below this limit were phakic (P = .01). The mean ketorolac level in the 7 eyes was 7.55 ng/mL (range, 5.0–14.9 ng/mL). The mean (SD) PGE₂ levels were 13.8 (3.8) pg/mL in control eyes and 11.7 (4.4) pg/mL in ketorolac-treated eyes (P = .04). Treatment with ketorolac resulted in a 15% reduction in PGE₂ levels. When only pseudophakic eyes were analyzed, mean (SD) PGE₂ levels were 14.1 (4.1) pg/mL in control eyes and 11.6 (4.5) pg/mL in ketorolac-treated eyes (P < .05).

CONCLUSIONS AND RELEVANCE Topical ketorolac 0.45% can obtain a vitreous level that exceeds its median inhibitory concentration and can significantly decrease vitreous PGE₂ levels. Vitreous levels of ketorolac were significantly higher in pseudophakic eyes than in phakic eyes. The results of this study suggest that topically administered ketorolac 0.45% may allow meaningful inhibition of prostaglandins in the retina.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01609881
N onsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed classes of medications owing to their analgesic, antipyretic, and anti-inflammatory properties. Potent inhibitors of cyclooxygenase (COX) enzymes and thereby the synthesis of all downstream proinflammatory prostaglandins, NSAIDs are used extensively in ophthalmology to stabilize pupillary dilation during intraocular surgery and to treat postoperative inflammation, pain, and cystoid macular edema. In addition to these established therapeutic applications, a growing body of scientific evidence suggests that NSAIDs may have therapeutic benefits in age-related macular degeneration (AMD), diabetic retinopathy (DR), and other retinal diseases.

Of all the commercially available ophthalmic NSAIDs, ketorolac tromethamine has the most studies supporting its safety and efficacy. Ketorolac 0.5% (Acular; Allergan Inc) and ketorolac 0.4% (Acular LS; Allergan Inc) are older formulations that have limited bioavailability in the vitreous after topical administration. In 2009, a preservative-free 0.45% formulation of ketorolac tromethamine (Acuvail; Allergan Inc) was approved by the Food and Drug Administration. The incorporation of carboxymethylcellulose as a vehicle considerably enhances the pharmacokinetics of this formulation.

To our knowledge, there are no published studies assessing the vitreous concentration of ketorolac 0.45% and its effect on vitreous prostaglandin 
E2 (PGE2) levels. Therefore, the primary intent of this study was to determine the vitreous levels of ketorolac 0.45% after topical application. Our secondary intent was to determine whether topical use of ketorolac 0.45% reduces PGE2 levels in the vitreous of patients.

### Methods

#### Study Population
The Vanderbilt University Institutional Review Board approved this study, and all patients gave informed consent before enrollment. The study complied with all aspects of the Health Insurance Portability and Accountability Act and was conducted in accord with the tenets of the Declaration of Helsinki. Patients were enrolled between June and December 2012.

All adult patients (aged ≥18 years) undergoing pars plana vitrectomy for macular hole, epiretinal membrane, or vitreous opacities were eligible for inclusion. The exclusion criteria were diabetes mellitus, previous pars plana vitrectomy in the study eye, prior intravitreal injection, coexistent retinovascular or other ocular inflammatory disease (eg, retinal arterial or venous occlusive disease and uveitis), history of ocular trauma, aphasis, presence of an anterior chamber intraocular lens, and topical use of NSAIDs or corticosteroids. Any history of systemic NSAID or corticosteroid use was recorded.

#### Study Groups
The first 12 consecutive eyes enrolled were assigned to treatment, and patients were instructed to apply 1 drop of ketorolac 0.45% 4 times daily, beginning 3 days before surgery, for a total of 12 doses. On the day of surgery, treated eyes were administered 3 drops of ketorolac 0.45% at 5-minute intervals beginning 1 hour before surgery. All patients were given samples of the drug and instructed to close their eye for 5 minutes after each application. The next 12 consecutive eyes served as controls and did not receive any treatment.

The recruitment objective of 24 patients was predetermined to allow vitreous samples to be tested in triplicate after accounting for standards and controls on a single 96-well plate. The use of a single plate, for prostaglandin testing, as opposed to multiple plates, maximizes the consistency and comparative reliability of specimen results taken from different patients. No patients in either group withdrew from the study.

#### Sample Collection
Vitrectomy surgery was performed using either a 23- or 25-gauge transconjunctival system. After placement of all 3 valved cannulas, 0.5 to 1.0 mL of undiluted vitreous was removed using the vitreous cutter positioned in the midvitreous cavity. Samples were immediately aliquoted into smaller tubes and frozen at −80°C.

#### Measurement of Prostaglandin Levels
At a later date, vitreous samples were thawed and PGE2 levels were analyzed using the Prostaglandin E2 Monoclonal EIA Kit (Cayman Chemical Company) according to the manufacturer’s instructions. In brief, serial dilutions of standards (7.8-1000 pg/mL) were prepared. Standards, controls, and vitreous samples were added to individual wells, followed by PGE2 acetylcholinesterase tracer and PGE2 monoclonal antibody. The plates were incubated overnight at 4°C. The plates were washed, and Ellman reagent and tracer were added to each well. The plates were covered and incubated at room temperature with gentle shaking for 100 minutes. The plates were read, and the data were analyzed using Microsoft Excel (Microsoft Corporation) and the Cayman Chemical Company EIA Triple workbook. The cross-reactivity between PGE2 and prostaglandin F2α is less than 0.01%.

#### Measurement of Ketorolac Levels
Sample analyses were carried out using a Surveyor high-performance liquid chromatography system (Thermo Fisher). Tandem mass spectrometric detection was performed using a TSQ Quantum triple-stage quadrupole mass spectrometer (Thermo Fisher). Quantitation was based on multiple-reaction monitoring detection of ketorolac. Internal standards were used. Data acquisition and quantitative spectral analysis were performed using Thermo Finnigan Xcalibur software (version 1.3) and Thermo Finnigan LCQuan software (version 2.70), respectively. Calibration curves were constructed, and the method was validated over the concentration range of 5 to 225 nmol/L (1.88-84.7 ng/mL). The lower limit of quantitation was approximately 3.3 ng/mL. Vitreous specimens that were not exposed to ketorolac were also tested in masked fashion as negative controls to verify the accuracy of results.
Statistical Analysis
Descriptive statistics, including means and SDs, were calculated for case characteristics. Group comparisons were performed with a t test using 2-sided analysis. The Fisher exact test was used to compare categorical values. Differences were considered statistically significant at \( P < .05 \). Multivariate analysis using logistic regression was performed to determine whether there was any confounding effect from independent variables (lens status, age, and systemic aspirin or NSAID use).

Results
Study Population
The study population consisted of 24 eyes of 22 patients. In 1 patient, both eyes were treated with ketorolac, and in a second patient 1 eye was treated with ketorolac and the other eye served as a control. The mean (SD) age was 67.5 (9) years in the ketorolac and 65.2 (10.3) years in the control group (\( P = .56 \)). Surgical indications were identical for both ketorolac and placebo groups: in each group, 10 eyes had epiretinal membrane, 1 eye had macular hole, and 1 eye had vitreous opacities. In the ketorolac group, 8 eyes were pseudophakic and 4 were phakic. In the control group, 9 eyes were pseudophakic and 3 were phakic. All pseudophakic eyes had posterior chamber intraocular lenses. The 2 groups were similar in their preoperative use of aspirin (3 eyes in the ketorolac and 4 in the control groups), systemic NSAIDs (0 eyes in the ketorolac and 1 in the control groups), and prednisone (1 eye in the control group at a dosage of 1 mg/d). Prostaglandin \( F_2\alpha \) analogs were used topically in 1 eye in the ketorolac group and 2 in the control group.

Vitreous Ketorolac Levels
Of the 12 eyes that received ketorolac, 7 (58%) had levels above the lower limit of quantitation (Table). All 7 eyes were pseudophakic (\( P = .01 \)). In these 7 eyes, the mean ketorolac concentration was 7.55 ng/mL (range, 5.0-14.9 ng/mL). Four of the 5 eyes that were below the lower limit of quantitation were phakic (both eyes enrolled from the same patient were phakic and below the lower limit of detection). No detectable ketorolac was found in the vitreous of control eyes that did not receive ketorolac.

Vitreous Prostaglandin Levels
The mean (SD) vitreous PGE\(_2\) levels were 13.8 (3.8) pg/mL (range, 7.2-26.2 pg/mL) in the control group and 11.7 (4.4) pg/mL (range, 1.3 to 17.5 pg/mL) in the ketorolac group (\( P = .04 \)). Overall, ketorolac lowered PGE\(_2\) levels by 15% (Figure). Among pseudophakic eyes, the mean (SD) PGE\(_2\) levels were 14.1 (4.1) and 11.6 (4.5) pg/mL in the control and ketorolac groups, respectively (\( P < .05 \)). Multivariate regression analysis did not show any significant confounding effect from lens status, patient age, or systemic aspirin or NSAID use.

Discussion
Accumulating scientific evidence suggests that inflammation has a pathogenic role in retinal disease.\(^4\)\(^5\) The results of our pilot study demonstrate that topical ketorolac 0.45% can attain vitreous levels sufficient to reduce vitreous PGE\(_2\) levels. To our knowledge, we are the first to report therapeutic vitreous levels of ketorolac above the median inhibitory concentration in patients after topical application. These results have clinical implications because the enhanced pharmacokinetics of ketorolac 0.45% may now allow meaningful inhibition of prostaglandins in the retina.

Cyclooxygenase is an important enzyme in the inflammatory process and catalyzes the biosynthesis of 5 classes (PGE\(_2\),...
Vitreous Prostaglandin E₂ With Ktorolac 0.45%

Original Investigation Research

prostaglandin D₂, prostaglandin F₂α, prostaglandin I₂, thromboxane A₂ of prostaglandins from arachidonic acid. The NSAIDs are potent inhibitors of COX enzymes and thereby the synthesis of prostaglandins (particularly PGE₂, which is more commonly associated with inflammation). Two isoforms, COX-1 and COX-2, are firmly established and are among the most thoroughly studied and best understood mammalian enzymes. In the human retina, COX enzyme can be detected in retinal endothelial cells, astrocytes, microglia, ganglion cells, amacrine cells, Müller cells, and retinal pigment epithelium cells and is upregulated in response to inflammatory cytokines. Within the eye, prostaglandins promote vasodilation, disrupt the blood-ocular barrier, facilitate leukocyte migration, and interact with and amplify many other soluble mediators.

Experimental evidence is considerable that prostaglandins have a direct role in the pathogenesis of AMD and DR. In a variety of experimental systems, inhibition of COX suppresses angiogenesis. The COX-2 enzymes have been detected in choroidal neovascular membranes, and pharmacologic inhibition or genetic deletion of COX reduces choroidal neovascularization in animal models. In animal models of DR, prostaglandins induce vascular endothelial growth factor production, with subsequent development of vascular leakage and retinal neovascularization. Levels of PGE₂ are increased by 40% in the retinal vasculature of diabetic rats and topical naprosyn 0.1% and celecoxib significantly inhibit diabetes-induced retinal microvascular disease and vascular leakage, respectively, in diabetic animals.

In addition to experimental evidence, several clinical studies have also reported therapeutic effects of NSAIDs for AMD and DR. Two recent randomized, prospective, clinical studies by Flaxel et al and Gomi et al found that topical bromfenac resulted in a greater reduction in macular thickness and a decreased frequency of anti-vascular endothelial growth factor treatment, respectively, in patients with exudative AMD. The level of PGE₂ is significantly elevated in the vitreous of patients with proliferative DR, and high-dose aspirin was found to slow the development of retinal microaneurysms. The NSAID sulindac prevented progression of DR in a 3-year controlled trial and oral celecoxib significantly reduced vascular leakage in patients with DR, despite the fact that treatment was stopped prematurely because of concerns regarding drug-related cardiovascular toxicity.

The potential toxicity (cardiovascular, renal, or gastrointestinal) of systemic NSAIDs underscores the obvious advantage of topical administration. To our knowledge, only 1 published study has demonstrated detectable vitreous levels of ketorolac after topical application, but that study used ketorolac 0.4%, and levels were below the median inhibitory concentration for both isoforms of COX. Ktorolac 0.45% is a newer, preservative-free 0.45% formulation of ketorolac in carboxymethylcellulose that has been approved for pain and inflammation after cataract surgery. The 12% higher concentration compared with the 0.4% formulation in combination with carboxymethylcellulose vehicle and lower pH result in significantly greater bioavailability in the aqueous humor. A study by Attar et al demonstrated that ketorolac 0.45% delivered 2-fold higher levels of ketorolac to the aqueous humor and 3-fold higher levels to the iris-ciliary body than the 0.4% formulation. In patients undergoing phacoemulsification, ketorolac 0.45% achieved greater inhibition of PGE₂ in the aqueous humor than naprosyn 0.1% and bromfenac 0.09%.

All of the commercially available ophthalmic NSAIDs, ketorolac has the most studies supporting its safety, positioning it more favorably for long-term administration. Our results demonstrate that ketorolac 0.45% reaches vitreous levels sufficient to inhibit its target enzyme and were 2.5-fold higher than reported with ketorolac 0.4%. Greater vitreous drug levels in pseudophakic eyes than in phakic eyes have been observed by others and are presumably due to disruption of zonular filaments and the posterior capsule/anterior hyaloid after phacoemulsification or laser capsulotomy. Although vitreous ketorolac levels were below the median inhibitory concentration for COX-2, the relative importance of COX-1 and COX-2 inhibition in retinal disease is unknown, and a significant decrease in vitreous PGE₂ concentration was still observed.

As with all pilot studies, our results should be interpreted with caution. Although the difference between PGE₂ levels in the vitreous of ketorolac 0.45%–treated and untreated eyes was significant, these levels are not a direct measure of retinal concentration. Nevertheless, it is generally accepted that vitreous levels correlate with retinal levels, and this method of analysis is widely accepted, given the unacceptable risks to patients with direct sampling of retinal tissue. Although patients were not randomized, baseline characteristics were similar between groups, and multivariate analysis did not demonstrate any confounding factors that could explain our observations. Furthermore, the enrollment of both eyes of 1 patient to ketorolac 0.45% treatment probably led to underestimation rather than overestimation of vitreous ketorolac levels because both eyes were phakic and drug levels were below the lower limits of detection.

In conclusion, our results demonstrate that ketorolac 0.45% can achieve sufficient vitreous levels to reduce vitreous PGE₂ levels, but drug bioavailability was significantly greater in pseudophakic eyes than in phakic eyes. These findings, if confirmed by larger independent studies, suggest that topical ketorolac may allow meaningful inhibition of retinal prostaglandins.
Conflict of Interest Disclosures: None reported.

Funding/Support: This work was supported by Research to Prevent Blindness (unrestricted grant to the Vanderbilt University School of Medicine Department of Ophthalmology and Visual Sciences) and by the National Center for Advancing Translation Sciences (CTSA award UL1TR000445).

Role of the Sponsor: The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES


24. Heier JS, Awh CC, Busbee BG, et al. Vitreous nonsteroidal antiinflammatory drug concentrations and prostaglandin E2 levels in vitrectomy patients treated with ketorolac 0.4%, bromfenac 0.09%, and nepafenac 0.1%. Retino. 2009;29(9):1310-1313.

