Reduction of Interleukin 8 and Platelet-Derived Growth Factor Levels by Topical Ketorolac, 0.45%, in Patients With Diabetic Retinopathy

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IMPORTANCE Inhibition of inflammatory cytokines may have therapeutic effects in diabetic retinopathy (DR).

OBJECTIVE To compare aqueous and vitreous levels of 17 inflammatory cytokines in patients treated preoperatively with topical ketorolac tromethamine, 0.45%, or placebo before pars plana vitrectomy for complications related to proliferative DR (PDR).

DESIGN, SETTING, AND PARTICIPANTS A prospective, randomized, placebo-controlled, patient-masked interventional study performed in a university academic hospital included 20 eyes from 20 patients undergoing pars plana vitrectomy for complications of PDR.

INTERVENTIONS Eyes were randomized to ketorolac tromethamine, 0.45% (Acuvail), or placebo 4 times daily for 3 days before pars plana vitrectomy. Undiluted aqueous and vitreous samples were taken at the time of surgery and immediately frozen at −80°C.

MAIN OUTCOMES AND MEASURES Aqueous and vitreous levels of prostaglandin E₂ and 16 other inflammatory cytokines implicated in the pathogenesis of DR.

RESULTS Prostaglandin E₂, platelet-derived growth factor (PDGF) AA, eotaxin, vascular endothelial growth factor, interferon γ-inducible protein of 10 kDa, monocyte chemoattractant protein 1, growth-related oncogene, interleukin 6, interleukin 8 (IL-8), and tumor necrosis factor were detectable in the aqueous and vitreous of at least half of the eyes, and these cytokines were analyzed further. Aqueous levels were lower in the ketorolac group for all cytokines detected, but only the difference in IL-8 was statistically significant (52% reduction; \( P = .04 \)). Levels of IL-8 (41% reduction; \( P = .002 \)) and PDGF-AA (21% reduction; \( P = .009 \)) were significantly lower in the vitreous of patients treated with ketorolac.

CONCLUSIONS AND RELEVANCE Topical ketorolac tromethamine, 0.45%, significantly lowered aqueous IL-8 levels and vitreous IL-8 and PDGF-AA levels in this series of eyes, suggesting that it may cause meaningful inhibition of inflammatory cytokines implicated in the pathogenesis of DR.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01609881
Diabetic retinopathy (DR) is a frequent cause of legal blindness. Proven preventable measures include strict control of blood glucose and lowering of high blood pressure, but there is increasing scientific evidence that inflammation plays a pathogenic role. In support of this, several studies have consistently shown elevated levels of inflammatory cytokines in the aqueous and vitreous of patients with proliferative DR (PDR) and diabetic macular edema.

Prostaglandins are an important class of inflammatory mediators that are biosynthesized from arachidonic acid by cyclooxygenase enzyme. Nonsteroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of this enzyme and thereby the synthesis of all downstream prostaglandins. Within the eye, prostaglandins increase vascular dilation, disrupt the blood-ocular barrier, and facilitate leukocyte migration. Consequently, their inhibition has favorable effects on intraocular inflammation and macular edema.

In experimental and animal models, prostaglandins induce vascular endothelial growth factor (VEGF) production with subsequent development of vascular leakage and retinal neovascularization. Prostaglandins are elevated in animal models of DR, and progression of retinopathy can be prevented or delayed with prostaglandin inhibitors. A recent pilot study reported elevated vitreous levels of prostaglandin E\(_2\) (PGE\(_2\)) in patients with PDR, and several prospective clinical studies have reported favorable effects on DR with systemic NSAIDs.

In 2009, ketorolac tromethamine, 0.45% (Acuvail; Allergan Inc), was approved for pain and inflammation after cataract surgery. Its enhanced pharmacokinetics enable significantly greater intraocular bioavailability than older formulations. The primary intent of this study was to determine the effect of ketorolac tromethamine, 0.45%, vs placebo on aqueous and vitreous levels of VEGF and 16 other inflammatory cytokines in diabetic patients undergoing vitrectomy surgery.

### Methods

#### Study Population

The Vanderbilt University Institutional Review Board approved this study, and all patients gave written informed consent. The study complied with all aspects of the Health Insurance Portability and Accountability Act and was conducted in accordance with the tenets of the Declaration of Helsinki. The trial is registered at clinicaltrials.gov (Identifier NCT01609881).

All adult patients (aged ≥18 years) undergoing pars plana vitrectomy for complications of PDR were eligible for inclusion. Exclusion criteria consisted of previous pars plana vitrectomy, prior intravitreal injection within 3 months, coexistent retinal disease (other than diabetes), and previous enrollment of the fellow eye. Preoperative use of topical and/or systemic anti-inflammatory medications was documented. Only patients with suspected or visible fibrovascular proliferation at the time of surgery were enrolled.

#### Study Groups

Patients were prospectively assigned by permuted block randomization to 1 of 2 groups. Group 1 (ketorolac) received single-use vials of topical ketorolac tromethamine, 0.45%, with all identifying trademark information removed, and group 2 (placebo) received single-use vials of preservative-free lubricating eye drops (Refresh Optive; Allergan Inc) prepared similarly. The vials of medication were identical in appearance and provided to patients in Acuvail packaging. All patients were instructed to apply 1 drop 4 times daily of their assigned drug, beginning 3 days before surgery, for a total of 12 doses. They also received 3 consecutive applications separated by 5 minutes, starting 1 hour before surgery.

The recruitment objective of 10 patients in each group was predetermined to allow vitreous samples to be tested in triplicate on a single 96-well multiplex cytokine plate for comparative reliability. All aqueous samples were tested once for cytokines owing to volume limitations.

#### Sample Collection

Vitrectomy was performed using 23- or 25-gauge systems. Approximately 0.1 mL of aqueous humor was removed using a 30-gauge needle, and approximately 0.5 to 1.0 mL of undiluted vitreous was removed by using the vitreous cutter in the midvitreous cavity before active infusion. The vitreous specimen was aliquoted into smaller tubes, and all samples were frozen at −80°C.

#### Measurement of Prostaglandin Levels

Aqueous and vitreous samples were thawed, and PGE\(_2\) levels were analyzed using the Prostaglandin E\(_2\) Monoclonal EIA Kit (Cayman Chemical Company) according to the manufacturer’s instructions, as described elsewhere. Testing was done in masked fashion.

#### Millipore Multiplex Kit

A microparticle bead-based multiplex assay was used to measure inflammatory cytokines in accordance with manufacturer’s instructions as described previously. In brief, samples were thawed and analyzed for the following 16 cytokines: eotaxin, Flt-3 ligand, growth-related oncogene, interferon (IFN) γ, interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12 (p40), IFN-γ inducible protein of 10 kDa, monocyte chemoattractant protein 1, tumor necrosis factor, VEGF, RANTES (regulated on activation of normal T expressed and secreted), and platelet-derived growth factor (PDGF) AA and AA/BB. Testing was done in masked fashion.

#### Statistical Analysis

Descriptive statistics, including means (patient age) and medians (cytokine levels), were calculated for case characteristics. Cytokines were statistically analyzed if at least half of the eyes had detectable values, and those with values below limits of detection were assigned a numerical value of 0 pg/mL for statistical analysis. Group comparisons were performed with the Wilcoxon rank sum test using 2-sided analysis. Categorical characteristics were analyzed using a Fisher exact test. Differences were considered statistically significant at \( P < .05 \).
Univariate and multivariate logistic regression analysis was performed to assess confounding effects from independent variables.

Results

Study Population
Twenty eyes from 20 patients were consecutively enrolled from July 2 to November 21, 2012. All patients underwent surgery for complications of PDR, including vitreous hemorrhage and/or tractional retinal detachments. Ten eyes were randomized to each group. Five additional patients were initially recruited but did not complete the study owing to cancellation of surgery (2 patients), noncompliance (2 patients), or insufficient sample collection (1 patient).

Baseline characteristics of both groups are shown in Table 1. The mean age in both groups was 52 years (P = .86). There were no significant differences between groups in terms of surgical indication, baseline lens status, use of insulin, duration of diabetes (<10 or ≥10 years), or systemic use of aspirin or NSAIDs. Significantly more eyes had received prior panretinal photocoagulation in the placebo group than in the ketorolac group (10 vs 5 eyes; P = .03). No patients in either group were being treated with systemic corticosteroids, systemic immunosuppression, topical corticosteroids, or prostaglandin analogues.

Aqueous Levels
Aqueous cytokine levels are summarized in Table 2. Ten cytokines were measurable in the aqueous humor of at least half of the eyes: PDGF-AA, VEGF, IFN-γ–inducible protein of 10 kDa, monocyte chemoattractant protein 1, GRO, platelet-derived growth factor AA, PGE₂, prostaglandin E₂, TNF, tumor necrosis factor, VEGF, vascular endothelial growth factor.

Vitreous Levels
Vitreous cytokine levels are summarized in Table 3. Ten cytokines were measurable in the vitreous of at least half of the
eyes: PDGF-AA, VEGF, IFN-γ-inducible protein of 10 kDa, monocyte chemoattractant protein 1, eotaxin, growth-related oncogene, IL-6, IL-8, tumor necrosis factor, and PGE2. Median PDGF-AA levels were 21% less in ketorolac-treated eyes than in placebo-treated eyes (654.5 vs 828.0 pg/mL; \(P = .009\); Figure 2). Similarly, median IL-8 levels were 41% less in ketorolac-treated eyes than in placebo-treated eyes (77.0 vs 131.0 pg/mL; \(P = .002\); Figure 3). Use of NSAIDs or aspirin was associated with a significant reduction in vitreous IL-8 levels (39% reduction; \(P < .01\)), but multivariate regression analysis demonstrated no significant confounding effects on comparison results between groups.

**Discussion**

Despite currently available treatments, DR remains a major cause of blindness worldwide. The results of our pilot study demonstrate that ketorolac significantly lowers vitreous IL-8 and PDGF-AA levels in patients with PDR. To our knowledge, we are the first to report this finding.

A growing body of scientific evidence indicates that inflammation contributes to the pathogenesis of DR.\(^5,20\) The retinal vasculature of diabetic humans contains increased numbers of leukocytes, and breakdown of the blood-ocular barrier begins early in its course.\(^21\) Both phenomena presumably lead to progressive dysfunction and death of endothelial cells and pericytes, which are pathophysiological hallmarks of DR. Subsequent capillary nonperfusion, retinal ischemia, and vascular leakage ensue, which are well-defined clinical features of DR. Cyclooxygenase enzyme is an enzyme in the inflammatory process and catalyzes the biosynthesis of 5 classes of pro-inflammatory prostaglandins (PGE\(_2\); prostaglandin D\(_2\), F\(_{2\alpha}\), and 12\(\)-thromboxane A\(_2\)) from arachidonic acid.\(^7\) In the human retina, cyclooxygenase enzyme can be detected in retinal endothelial cells, astrocytes, microglia, ganglion cells, amacrine cells, Müller cells, and retinal pigment epithelial cells and is upregulated in response to various inflammatory cytokines.\(^4,7,20\) Within the eye, prostaglandins promote vasodilation, disrupt the blood-ocular barrier, facilitate leukocyte migration, and interact with and amplify other soluble mediators.\(^20,22\)

Nonsteroidal anti-inflammatory drugs are potent inhibitors of cyclooxygenase enzyme and prevent or slow DR progression in a variety of experimental systems. In vitro studies demonstrate that PGE\(_2\) increases VEGF expression in cultured Müller cells, and agonism or antagonism of the PGE\(_2\) receptor EP\(_4\) increases or decreases VEGF production,
Figure 3. Vitreous Interleukin 8 (IL-8) Levels

Values are shown as median (horizontal line within box), interquartile range (box), and full range (whiskers).

respectively.\textsuperscript{9,10} Prostaglandin E\textsubscript{2} levels are increased by 40% in the retinal vasculature of diabetic rats and are significantly reduced by treatment with insulin.\textsuperscript{11} The NSAID celecoxib inhibits PGE\textsubscript{2} secretion, retinal VEGF expression, and vascular leakage in streptozotocin-induced diabetic rats,\textsuperscript{14,23} and nepadac, aspirin, and meloxicam have independently been reported to inhibit diabetes-induced retinal microvascular disease.\textsuperscript{12,13}

In addition to experimental evidence, there is emerging clinical evidence supporting a therapeutic effect of NSAIDs in DR. Studies have consistently shown elevated levels of several proinflammatory cytokines in the aqueous and vitreous of patients with PDR and diabetic macular edema.\textsuperscript{4,6,24} The Dipyridamole Aspirin Microangiopathy of Diabetes Study\textsuperscript{45} found that high doses of aspirin significantly slowed the development of retinal microaneurysms, and this observation was supported by a randomized 3-year study that showed similar delays in diabetic patients treated with the NSAID sulindac.\textsuperscript{46} More recently, a prospective controlled trial conducted by the National Eye Institute demonstrated that oral celecoxib reduced vascular leakage in patients with DR despite premature stoppage of treatment due to concerns regarding cardiovascular toxic effects.\textsuperscript{17}

Topical administration minimizes systemic exposure and, in recent years, topical NSAIDs have been formulated with enhanced intraocular penetration. Of all the commercially available ophthalmic NSAIDs, ketorolac has the most studies supporting its safety.\textsuperscript{7,8} Ketonolac tromethamine, 0.45%, is preservative free\textsuperscript{48} and achieves 2.5-fold higher vitreous levels than older formulations.\textsuperscript{25} Its excellent safety, enhanced bioavailability, and lack of preservatives make it well suited for long-term application.

It is possible that IL-8 and PDGF-AA contribute to the pathogenesis of DR. Interleukin 8 is a potent chemoattractant and activator of neutrophils\textsuperscript{26} and one of the most consistently reported cytokines to have elevated levels in the plasma and vitreous of diabetic patients.\textsuperscript{4,27} Exposure to PGE\textsubscript{2} directly induces IL-8 gene transcription in human T cells,\textsuperscript{28} and human retinal pigment epithelial cells secrete IL-8 in direct response to proinflammatory cytokines (IL-1\textbeta, tumor necrosis factor, and IFN-\gamma).\textsuperscript{29} Within the eye, IL-8 promotes angiogenesis, induces endothelial cell production of peroxides and superoxides, and disrupts the blood-ocular barrier.\textsuperscript{26,23} This latter action may propagate and amplify other downstream inflammatory pathways.

Platelet-derived growth factor plays an important role in wound healing and angiogenesis.\textsuperscript{32} It is a strong chemoattractant and mitogen for mesenchyme-derived cells and glial cells and promotes pericyte migration and synthesis and deposition of collagen by fibroblasts.\textsuperscript{29} Although present in a wide variety of cell types, the main sources of PDGF in the retina seem to be retinal pigment epithelial cells, astrocytes, and ganglion cells.\textsuperscript{33,34} Three main isoforms exist (AA, AB, and BB), and considerable scientific evidence indicates that PDGF directly contributes to neovascularization and fibrovascular proliferation in PDR.\textsuperscript{35,36} In support of this, PDGF-AA levels are consistently elevated in the aqueous and vitreous of patients with active PDR\textsuperscript{4,6,32} and seem to be reduced in patients after panretinal photoagulation or treatment with corticosteroids.\textsuperscript{35,36}

As with all pilot studies, our results should be interpreted with caution. Because of the small number of patients in this study, there were baseline differences between groups. For example, a higher proportion of patients treated with placebo had undergone prior panretinal photoagulation, but this variance would be more likely to lead to underestimation rather than overestimation of our results because panretinal photoagulation treatment would probably minimize any observed treatment effect of ketorolac. Despite the study’s limitations, we would like to emphasize its strengths, which include its prospective randomized design.

In conclusion, our results demonstrate that topical ketorolac tromethamine, 0.45%, significantly reduces vitreous levels of IL-8 and PDGF-AA in patients with PDR. To our knowledge, this is the first study to provide direct clinical evidence that topical NSAIDs may decrease posterior segment inflammation. These findings, if substantiated by other larger studies, provide a rationale for anti-inflammatory–based therapies to prevent or delay progression of DR.
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REFERENCES