REDUCTION OF VITREOUS PROSTAGLANDIN E\(_2\) LEVELS AFTER TOPICAL ADMINISTRATION OF INDOMETHACIN 0.5%, BROMFENAC 0.09%, AND NEPAFENAC 0.1%

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**Purpose:** To assess vitreous concentrations of nonsteroidal anti-inflammatory drugs (NSAIDs) and prostaglandin E\(_2\) (PGE\(_2\)) in patients treated with NSAIDs before vitrectomy for macular pucker.

**Methods:** A prospective, investigator-masked, randomized study was performed in 64 patients scheduled to undergo vitrectomy. The patients were randomized 1:1:1:1 to receive indomethacin 0.5%, bromfenac 0.09%, nepafenac 0.1%, or placebo three times a day. NSAIDs and PGE\(_2\) levels were evaluated in vitreous samples collected at the beginning of surgery.

**Results:** Mean (SD) vitreous concentrations of the study drugs were 503.13 (241.1) pg/mL for indomethacin, 302.5 (91.03) pg/mL for bromfenac, and 284.38 (128.2) pg/mL for nepafenac. Mean (SD) vitreous PGE\(_2\) levels were 247.9 (140.9) pg/mL for indomethacin, 322.12 (228.1) pg/mL for bromfenac, 448.8 (261.1) pg/mL for nepafenac, and 1,133 (323.9) pg/mL for placebo. All three NSAIDs reduced vitreous PGE\(_2\) levels to a statistically significant extent, without a significant difference among them.

**Conclusion:** All assessed NSAIDs penetrated the vitreous and lowered basal PGE\(_2\) levels. A greater penetration was associated with pseudophakic eyes. The important inhibition of prostaglandins in the retina may have a clinical effect on the management of inflammatory retina diseases.

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed classes of medication, and they are routinely used for their analgesic, antipyretic, and anti-inflammatory properties. Because they are potent inhibitors of cyclooxygenase (COX) enzymes, they reduce the synthesis of proinflammatory prostaglandins. Nonsteroidal anti-inflammatory drugs have been extensively used systemically for many decades and have more recently become available in various types of topical ophthalmic formulations.\(^1\) Nonsteroidal anti-inflammatory drugs are widely used in the field of ophthalmology to stabilize pupillary dilation during intraocular surgery and to treat postoperative inflammation, pain, and cystoid macular edema.\(^2\) A growing body of evidence suggests that NSAIDs may also be beneficial for treating age-related macular degeneration (AMD), diabetic retinopathy, and ocular tumors.\(^3\)–\(^6\)

Although topical administration of NSAIDs has been widely shown to achieve therapeutic levels in the aqueous humor,\(^7\) this therapeutic effect is less evident in the retina and the choroid. Knowing the extent of NSAID penetration into the vitreous humor and the...
consequent level of inflammatory mediators, such as prostaglandin E2 (PGE2), may be of clinical interest for the treatment of posterior segment diseases. This study aimed to assess vitreous concentrations of indomethacin 0.5%, bromfenac 0.09%, and nepafenac 0.1%, and PGE2 levels after topical administration in patients undergoing vitrectomy for macular pucker.

Materials and Methods

This was a prospective, investigator-masked, randomized study in eyes scheduled to undergo 25-gauge, 3-port pars plana vitrectomy for macular pucker. This study was conducted at the University Eye Clinic of “Spedali Civili di Brescia,” in accordance with the ethical principles of the Declaration of Helsinki. The ethics committee of Spedali Civili di Brescia (Italy) approved the study protocol (registered with ClinicalTrials.gov, identifier NCT02361645). All study participants provided written informed consent.

All patients ≥18 years undergoing scheduled pars plana vitrectomy for macular pucker or vitreous macular adhesion syndrome were eligible for inclusion. The exclusion criteria were vitreous hemorrhage, diabetes mellitus, previous vitrectomy in the study eye, previous intravitreal injection, concurrent retinovascular or other ocular inflammatory disease, history of ocular trauma, and concomitant intake of any topical or systemic NSAID or corticosteroid therapy.

Sixty-four patients were randomized 1:1:1:1 to receive indomethacin 0.5%, bromfenac 0.09%, nepafenac 0.1%, or placebo three times a day. All patients received the study drugs for 7 days before surgery. The placebo group received single-use vials of hyaluronic acid 0.2% preservative-free lubricating eye drops and served as a control group.

Vitrectomy surgery was performed using a 25-gauge transconjunctival system. After placement of all three pars plana trocars, 0.5 mL to 1.0 mL of undiluted vitreous was removed from the midvitreous cavity, just in front of the posterior pole, at the beginning of the surgery before infusion with balanced salt solution. Samples were immediately frozen and stored at −40°C until analysis.

Measurements of Prostaglandin Levels

Vitreous samples were defrosted and subjected to semiquantitative determination of PGE2 levels. Analyses were performed using Dynex Technologies DSX (Software Version 6.03; Chantilly, VA) according to manufacturer’s instructions. Briefly, analysis was based on the competition between PGE2 and PGE2-acetylcholinesterase conjugate (PGE2 tracer) for a limited amount of PGE2 monoclonal antibody. Because the concentration of the PGE2 tracer was constant while the concentration of PGE2 samples varied, the amount of PGE2 tracer, which was able to bind to the PGE2 monoclonal antibody, is inversely proportional to the concentration of PGE2 in the well.

Measurements of Nonsteroidal Anti-Inflammatory Drugs Levels

Sample analyses were performed using an HPLC 1260 System (Agilent Technologies, Santa Clara, CA) equipped with an infinity system model 1260 injector and paired with a 6460 Triple Quad LC/MS triple quadruple mass spectrometer (Agilent Technologies).

Chromatographic separation of NSAIDs was achieved using Kinetex (Phenomenex, Torrance, CA) pentfluoropropionic (PFP) 100 mm × 2.10 mm, 2.6 μm coupled with a security guard column. The flow rate was 0.2 mL/minute with a mobile phase of 0.01% formic acid in water and acetonitrile. The total run time was 15 minutes per sample. In total, 200 μL of vitreous humor were added into glass test tubes with hermetic screw caps, vortexed, and incubated on a rotating agitator for 15 minutes at room temperature. After centrifugation at 4,000 rpm for 10 minutes at 4°C, the samples were transferred to vials with screw tops and 10 μL was directly injected into the LC–MS/MS (liquid chromatography-mass spectrometry) system. Calibration curves were constructed, and the method was validated over a concentration range of 0.025 ng/mL to 10 ng/mL.

Statistical Analyses

Descriptive statistics are used to present demographic and ocular baseline characteristics. A one-way analysis of variance was conducted to analyze differences in PGE2 levels between the treatment arms. The sample size of 64 patients provided a power of 0.99, for demonstrating an effect size of 1.85 between the 4 groups, at a significance level of 0.05. An independent samples t-test was run to determine whether there were differences in drug concentration between phakic and pseudophakic eyes. All statistical analyses were performed using SPSS software V.20 (IBM Corp, Armonk, NY). P < 0.05 was considered significant.

Results

Sixty-four eyes of 64 patients were consecutively enrolled from February to October 2014 and completed the study. Sixteen eyes were randomized in each group. Demographics characteristics of enrolled patients are listed in Table 1.
Mean (SD) vitreous concentrations of the study drugs were 503.13 (241.1) pg/mL for indomethacin, 302.5 (91.03) pg/mL for bromfenac, and 284.38 (128.2) pg/mL for nepafenac. Mean (SD) vitreous PGE$_2$ levels were 247.9 (140.9) pg/mL for indomethacin, 322.12 (228.1) pg/mL for bromfenac, 448.8 (261.1) pg/mL for nepafenac, and 1,133 (323.9) pg/mL for placebo. The decrease in vitreous PGE$_2$ levels was statistically significant for all the NSAIDs (P, 0.0001), but no statistically significant difference was found between the assessed NSAIDs. Table 2 shows the vitreous concentrations of NSAIDs and PGE$_2$.

Vitreous concentrations of the study drugs significantly differed between phakic and pseudophakic eyes (Figure 1). Mean concentration (SD) of NSAIDs were as follows: indomethacin 358.6 (83.2) pg/mL and 615.6 (267) pg/mL (P = 0.029), bromfenac 234.3 (47.2) pg/mL and 355.6 (81.4) pg/mL (P = 0.004), and nepafenac 183.8 (35.8) pg/mL and 385.0 (103.9) pg/mL (0.001), respectively, for phakic and pseudophakic eyes.

**Discussion**

Results from this study showed that all assessed NSAIDs penetrated into the vitreous, reaching vitreous level sufficient to reduce vitreous PGE$_2$ levels. To our knowledge, we are the first to report therapeutic vitreous levels of indomethacin above the median inhibitory concentration in humans after topical administration. The indomethacin group showed the greater reduction of PGE$_2$ levels than the bromfenac and nepafenac groups, although this difference was not significant. Such results disclose clinical implications because the pharmacokinetics of the assessed NSAIDs allow for therapeutic anti-inflammatory use in the retina.

The COX enzyme, which is the target of NSAIDs, is widely expressed in the human retina, particularly in retinal pigment epithelium, endothelial cells, Müller cells, astrocytes, microglia, ganglion cells, and amacrine cells. Moreover, COX is upregulated as a consequence of the inflammatory cascade triggered by inflammatory cytokines$^{8,9}$ and is responsible for the biosynthesis of five classes of prostaglandins from arachidonic acid: prostaglandin D$_2$, prostaglandin F$_2$α, prostaglandin I$_2$, thromboxane A$_2$, and PGE$_2$, the latter of which is more commonly associated with inflammation.$^{10}$ Inside the eye, prostaglandins stimulate blood-ocular barrier disruption, vasodilation, facilitate leukocyte chemotaxis, and increase the production of inflammatory proteins and vascular endothelial growth factor (VEGF).$^{8,11}$ Indeed, a growing body of evidence is highlighting the direct role of prostaglandins in the pathogenesis of AMD and diabetic retinopathy.$^{10,12}$

Our results are consistent with those of a previous study by Heier et al$^{13}$ assessing the vitreous penetration of ketorolac, bromfenac, and nepafenac. These authors reported a significant penetration of all ophthalmic solutions into the vitreous cavity. However, they described significant PGE$_2$ reduction only in patients treated with ketorolac. This could be explained by the 80% prevalence of retinal detachments in their control group, as this condition leads to an increase of PGE$_2$ levels in the vitreous of patients with retinal detachment.$^{14}$

Schoenberger et al$^{10}$ recently described only a 15% reduction in PGE$_2$ levels in eyes treated with topical ketorolac tromethamine 0.45%. Although a direct comparison with this study is not possible, the greater reduction we reported could be probably explained with a longer period of treatment (7 vs. 3 days) and with different study NSAIDs.

| Drug vitreous concentration (SD), pg/mL | Indomethacin 0.5% | Bromfenac 0.09% | Nepafenac 0.1% | Placebo |
|---------------------------------------|-------------------|-----------------|---------------|---------|
| PGE$_2$ levels, pg/mL                 | 503.13 (241.1)    | 302.5 (91.03)   | 284.38 (128.2) | —       |
| P                                    | 0.832*            | 0.111*          | <0.0001†      |         |

*Refers to vitreous PGE$_2$ levels compared with indomethacin.
†Refers to placebo PGE$_2$ levels compared with all three study NSAIDs.
The therapeutic use of NSAIDs in the retina has been reported in both experimental and clinical studies, with a recent focus on the synergic effect with anti-VEGF therapies. Experimental studies have reported that COX enzyme inhibition can increase the efficacy of antiangiogenic treatments by suppressing VEGF and angiogenesis. More specifically, animal models show that pharmacologic inhibition or genetic deletion of COX improves choroidal neovascularization and diabetic retinopathy. Indeed, PGE2 stimulates VEGF expression in cultured rat Müller cells and PGE2 levels are significantly higher in the vitreous of patients with proliferative diabetic retinopathy complications, which correlates with VEGF levels. Bucolo et al assessed the effects of topical 0.5% indomethacin, 0.09% bromfenac, and 0.1% nepafenac in rats with endotoxin-induced uveitis. Consistent with our results, they reported significant inhibition of PGE2 with all NSAIDs and also showed a higher effect with indomethacin and bromfenac than with nepafenac.

Clinical trials in humans are consistent with evidence from experimental models, highlighting the clinical implications of the synergy between NSAIDs with anti-VEGF for the treatment of exudative AMD. Two prospective clinical studies by Flaxel et al and Gomi et al reported superior reduction in central retinal thickness (CRT) and reduced frequency of anti-VEGF injections in naïve patients with exudative AMD treated with topical bromfenac. Conversely, only a mild trend toward improved anatomy was reported after nepafenac combined with anti-VEGF treatment, although this was assessed in eyes with recalcitrant exudative AMD.

Herein, we observed significantly greater drug penetration in pseudophakic eyes compared with phakic eyes. This is in accordance with previous studies assessing the vitreous concentrations of topically administered drugs. The superior vitreous penetration is probably due to the disruption of the zonular filaments with phacoemulsification and the opening of the posterior capsule and anterior hyaloid after laser capsulotomy. Moreover, the implanted intraocular lens has a smaller diameter and volume compared with the natural lens.

There are a few limitations to this study. First, we only assessed the prodrug nepafenac and not its active metabolite amfenac. Nevertheless, this did not influence PGE2 level inhibition induced by nepafenac. Second, PGE2 concentrations in the vitreous are not a direct measure of retinal levels. However, since direct sampling of retinal tissue is not feasible without serious potential risks, vitreous levels are generally accepted to correlate with retinal levels. Third, this sample size is limited to address significant differences between the three assessed NSAIDs, although no difference was found.

In conclusion, our results demonstrate that indomethacin 0.5%, bromfenac 0.09%, and nepafenac 0.1% reach vitreous levels sufficient to reduce vitreous...
PGE\(_2\) levels compared with the control group in patients undergoing vitrectomy. Moreover, drug bioavailability was significantly higher in pseudophakic eyes than in phakic eyes. Additional larger, independent studies are warranted to corroborate our results because clinical implications arise from the therapeutic use of topical NSAIDs in the retina.

**Key words:** bromfenac, indomethacin, nepafenac, nonsteroidal anti-inflammatory drugs, prostaglandin E2, vitrectomy, vitreous.

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