VITREOUS PROSTAGLANDIN E_2 CHANGES AFTER TOPICAL ADMINISTRATION OF DICLOFENAC 0.1%, INDOMETHACIN 0.5%, NEPAFENAC 0.3%, AND BROMFENAC 0.09%

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Purpose: To evaluate the vitreous concentration of different nonsteroidal anti-inflammatory drugs (NSAIDs) after topical administration and the related prostaglandin E_2 (PGE_2) levels in patients undergoing pars plana vitrectomy.

Methods: A prospective, randomized, investigator-masked study was performed. One hundred four patients scheduled for a pars plana vitrectomy for an epiretinal membrane or a macular hole were randomized to receive topical diclofenac 0.1%, indomethacin 0.5%, nepafenac 0.3%, bromfenac 0.09%, or placebo 3 days before surgery. At the beginning of surgery, a sample of undiluted vitreous was collected in each patient to assess NSAIDs concentration and PGE_2 levels.

Results: The median vitreous concentrations were 203.35 (interquartile range 146.54–264.18) pg/mL for diclofenac, 243.45 (interquartile range 156.96–365.37) pg/mL for nepafenac, 438.21 pg/mL (interquartile range, 282.52–645.87) for its active metabolite amfenac, 350.14 (interquartile range, 290.88–481.95) pg/mL for indomethacin, and 274.59 (245.43–358.25) pg/mL for bromfenac. Vitreous PGE_2 levels were significantly lower for all the NSAIDs groups compared with the control group ($P < 0.001$). A statistically significant higher vitreous PGE_2 level was found in the diclofenac group compared with the other NSAIDs groups ($P < 0.05$).

Conclusion: Topical NSAIDs achieve sufficient vitreous concentration to decrease vitreous PGE_2 levels compared with the control group. The different efficacy in reducing PGE_2 concentration may affect the management of posterior segment inflammation.

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are a diffuse class of medications, commonly prescribed owing to their demonstrated analgesic, antipyretic, and anti-inflammatory properties.[1]

In ophthalmology, NSAIDs were initially approved to prevent the intraoperative miosis and later became a proven and effective treatment to reduce perioperative inflammation and pain in cataract and corneal refractive surgery.[2,3]

Mechanical damage related to surgical trauma leads to the production of phospholipase A2 and arachidonic acid from the cell membrane. The arachidonic acid is metabolized by cyclooxygenase (COX) enzyme to thromboxane (TX) and prostaglandins (PGs), which are important mediators of the inflammatory response.[4,5]

With different pharmacokinetics profiles, NSAIDs inhibit COX and prevent the biosynthesis of the PGs, which is responsible for anterior and posterior ocular inflammation, intraocular pressure changes, ocular pain, and miosis.[6,7]

Recently, several scientific evidences suggest a role of PGs in the pathogenesis of diabetic retinopathy, age-related macular degeneration, intraocular tumors, and other retinal diseases.[8–13]

Although the NSAIDs bioavailability in the aqueous humor has been widely demonstrated,[14–17] the

achievement of therapeutic levels in the posterior segment is less evident and still debated. The effect of NSAIDs in preventing posterior segment inflammation could be argued by dosing both the NSAID and prostaglandin E_2 (PGE_2) concentration into the vitreous after topical administration.

To the best of our knowledge, no studies are reporting the vitreous concentration of diclofenac 0.1% and nepafenac 0.3% after topical administration and their effect on vitreous PGE_2 levels in human eyes.

This study was conducted to evaluate the vitreous concentrations of four different NSAIDs (diclofenac 0.1%, indomethacin 0.5%, nepafenac 0.3%, and bromfenac 0.09%) and the related PGE_2 levels after topical administration in patients undergoing vitrectomy for idiopathic epiretinal membrane (ERM) or full-thickness macular hole.

Materials and Methods

Study Design

A prospective, randomized, investigator-masked study was conducted at the Eye Clinic of University of Trieste, in eyes scheduled for pars plana vitrectomy (PPV) for ERM or full-thickness macular hole. The study was performed according to the Italian bioethical legislation and followed the Declaration of Helsinki and Good Clinical Practice for research involving human subjects. The ethics committee of Azienda Sanitaria Universitaria Integrata di Trieste (C.E.U.R., Italy) approved the study protocol and registered with ClinicalTrials.gov (identifiers, NCT03597867). Written informed consent was obtained from all participants.

All patients aged 18 years and older undergoing PPV for ERM or MH were eligible for the study. Eyes with a pre-existing condition that could influence basal PGE_2 vitreous levels and concentration of administered NSAID were excluded. Therefore, the exclusion criteria were a history of retinal detachment, ischemic maculopathy, central or branch retinal vein occlusion, central or branch retinal artery occlusion, glaucoma, age-related macular degeneration, diabetic retinopathy, chronic or recurrent inflammatory eye disease, previous PPV or intravitreal injection, ocular trauma, aphakia, presence of an anterior chamber intraocular lens, use of topical ophthalmic PGs, systemic or topical, periocular, intraocular corticosteroids, or NSAIDs therapy in the six months before surgery. Patients allergic or hypersensitive to NSAIDs also were excluded from the study.

One hundred and four patients were randomized to receive diclofenac 0.1%, indomethacin 0.5%, nepafenac 0.3%, bromfenac 0.09%, or placebo. The study drugs were administered as per the own dosing regimens 4 times a day for diclofenac 0.1%, 3 times a day for indomethacin 0.5%, once a day for nepafenac 0.3%, and twice a day for bromfenac 0.09%. In the placebo group, single-use vials of 0.2% hyaluronic acid preservative-free lubricating eyedrops were administered twice a day and served as a control group. All patients received the study drugs for 3 days before surgery and were instructed to close their eye for 5 minutes after each application. On the day of the surgery, patients received a single drop of assigned treatment 1 hour before surgery.

Sample Collection

A conventional 3-port PPV was performed using a 27-gauge transconjunctival system under retrobulbar anesthesia (Constellation Vision System; Alcon, Fort-Worth-TX). At the beginning of the surgery, before starting the infusion with balanced salt solution, 0.5 mL to 1.0 mL of undiluted vitreous was collected from the midvitreous cavity, just in front of the posterior pole. Samples were immediately snap-frozen and stored at $-60°C$ until analysis.

Measurement of Prostaglandin Levels

Vitreous samples were thawed and subjected to quantitative analysis of PGE_2 levels using a commercially available Human Prostaglandin E_2 ELISA Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. In brief, the assay is based on the competition between PGE_2 and a PGE_2-alkaline phosphatize tracer for a limited amount of PGE_2-specific monoclonal antibody. Because of the competition between PGE_2 in the sample and PGE_2 tracer for the PGE_2 antibody, the signal obtained with the assay is inversely proportional to the amount of PGE_2 in each sample. Up to eight serial dilutions of standards were prepared (31–4,000 pg/mL). All samples were before purified and concentrated by a solid-phase extraction using SPE C-18 cartridges. Then, the equilibration was performed in the wells of a 96-well microtiter plate

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precoated with goat polyclonal anti-mouse IgG, which binds all of the PGE_2 monoclonal antibody added to the well. The plates were incubated overnight. After the equilibration step, the plate is washed and a solution of *para*-nitrophenyl phosphate, a substrate for alkaline phosphate, is added. The products of this enzymatic reaction were read at 412 nm, and the data were analyzed using Microsoft Excel (Microsoft Corporation).

Measurement of Nonsteroidal Anti-inflammatory Drug Levels

Samples analyses were conducted using an ultra–high-performance liquid chromatography (UHPLC ExionLC AD System; Sciex, Framingham, MA) coupled with a tandem mass spectrometer (QTrap 6500+; Sciex). Chromatographic separation was achieved using an Hypersil GOLD (Thermo Fisher Scientific, Waltham, MA) 50 mm × 2.1 mm, 3 μm at the flow rate of 0.6 mL/minute with a mobile phase A of 0.1% formic acid in water and phase B of 0.1% formic acid in acetonitrile.

After vortexing at the max speed of 14,500 rpm, 50 μL of the sample was added to 40 μL of the internal standard and vortexed again. After centrifugation for 10 minutes, 40 μL of the supernatant was diluted with 200 μL of mobile phase B and then injected into the LC-MS/MS system. Detection was achieved by multiple reaction monitoring of the m/z transitions 334 → 288; 255 → 210; 256 → 210; 296 → 214; 358 → 139; 240 → 184 for bromfenac, nepafenac, amfenac, diclofenac, indomethacin, and bupropion (internal standard), respectively, under electrospray ionization (ESI, positive mode). Calibration curves were constructed, and the method was validated over a concentration range of 0.025 ng/mL to 10 ng/mL. Data acquisition and quantitative analysis were performed using Analyst software (version 1.6.3).

Statistical Analysis

Descriptive statistics are used to present demographic and ocular baseline characteristics. This study was designed to show the reduction of prostaglandin levels among five groups of treatments. Assuming an effect size of 0.4, at least 20 eyes in each group were required for an 80% power and 0.05% significance level. The quantitative variables were summarized as mean and SD or median and interquartile range (IQR) according to their distribution. Qualitative variables were summarized as frequency and percentage. The Shapiro–Wilk test was performed to evaluate the departures from normality distribution for each variable. The Kruskal–Wallis test with post hoc pairwise comparison was performed to evaluate differences of PGE_2 concentration among therapy groups. The Mann–Whitney *U* test was assessed to evaluate the difference of drug concentrations between phakic and pseudophakic patients. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS software 11.0 (SPSS Inc, Chicago, IL).

Results

The study population consisted of 104 eyes of 104 patients. Demographics of enrolled patients are listed in Table 1. Forty-three patients were men (41.4%) and 61, women (58.6%), with age ranging from 57 to 84 years. Fifty of 104 patients (48%) were already pseudophakic. All pseudophakic eyes had posterior chamber intraocular lenses. There were no between-group differences in sex ($P = 0.158$), age ($P = 0.203$), or proportion of pseudophakic patients ($P = 0.176$). Surgical indications were ERM in 83 of 104 eyes (79.8%). Four MH were included in each group, apart from the indomethacin 0.5% group in which 5 MH were enrolled.

The median vitreous concentrations of the tested NSAIDs were 203.35 (IQR 146.54–264.18) pg/mL for diclofenac, 243.45 (IQR 156.96–365.37) pg/mL for nepafenac, 438.21 pg/mL (IQR, 282.52–645.87) for its active metabolite amfenac, 350.14 (IQR, 290.88–481.95) pg/mL for indomethacin, and 274.59 (245.43–358.25) pg/mL for bromfenac. No detectable NSAIDs were found in the vitreous of control eyes that did not receive the study drugs.

Table 1. Baseline Demographic Characteristics of the Study Cohort

Characteristics	Diclofenac 0.1%	Nepafenac 0.3%	Indomethacin 0.5%	Bromfenac 0.09%	Placebo
Patient, n	20	21	22	21	20
Sex (male:female)	8:12	9:12	9:13	8:13	9:11
Mean age, SD (years)	73.2 (8.4)	68.5 (10.1)	71.7 (7.8)	75.1 (8.1)	71.9 (8.7)
Lens status					
Phakic	10	11	12	11	10
Pseudophakic	10	10	10	10	10

Table 2. Vitreous Concentrations of NSAIDs and Related PGE_2 Levels

	Diclofenac 0.1%	Nepafenac 0.3%	Indomethacin 0.5%	Bromfenac 0.09%	Placebo
NSAID vitreous concentration (IQR), pg/mL	203.35 (146.54–264.18)	243.45* (156.96–365.37)	350.14 (290.88–481.95)	274.59 (245.43–358.25)	–
PGE2 levels (IQR), pg/mL	2,066.95 (1,511.41–2,467.59)	1,640.79 (1,432.02–1,891.86)	1,592.86 (1,270.27–1,888.73)	1,596.42 (1,278.51–1,818.17)	3,262.60 (3,080.22–3,463.06)
P	–	0.013†	0.004†	0.004†	<0.001‡

*Active metabolite amfenac concentration: 438.21 pg/mL (IQR, 282.52–645.87).
†Pairwise analysis of vitreous PGE_2 levels compared with diclofenac 0.1%.
‡Pairwise analysis of vitreous PGE_2 levels compared with all tested NSAIDs.

The median vitreous PGE_2 levels were 3,262.60 (IQR 3,080.22–3,463.06) pg/mL in the placebo group, 2,066.95 (IQR 1,511.41–2,467.59) pg/mL in the diclofenac group, 1,640.79 (IQR 1,432.02–1,891.86) pg/mL in the nepafenac group, 1,592.86 (IQR 1,270.27–1,888.73) pg/mL in the indomethacin group, and 1,596.42 (IQR 1,278.51–1,818.17) pg/mL in the bromfenac group (Figure 1). The decrease in vitreous PGE_2 levels was statistically significant for all the NSAIDs compared with the control group ($P < 0.001$), and a statistically significant difference was found between the diclofenac group and other assessed study drugs ($P = 0.013$ for diclofenac vs. nepafenac; $P = 0.004$ for diclofenac vs. indomethacin or bromfenac). The vitreous concentrations of the different NSAIDs and related PGE_2 levels were reported in Table 2.

According to the lens status, a statistically significant difference ($P < 0.001$) in PGE_2 levels was found between the phakic and pseudophakic subgroups (Figure 2). The median diclofenac vitreous concentrations were 145.26 (IQR 121.89–181.29) pg/mL in phakic eyes and 266.81 (IQR 246.97–308.22) pg/mL in pseudophakic eyes. Nepafenac vitreous concentrations were 153.81 (IQR 138.51–177.20) pg/mL in phakic eyes and 365.37 (IQR 319.34–396.24) pg/mL in pseudophakic eyes. Amfenac vitreous concentration were 273.44 (IQR 249.75–329.90) pg/mL and 645.87 (IQR 539.56–705.25) pg/mL in phakic and pseudophakic eyes, respectively. Indomethacin vitreous concentrations were 287.56 (227.33–320.77) pg/mL in phakic eyes and 485.14 (378.45–593.46) pg/mL in pseudophakic eyes. Finally, bromfenac vitreous concentrations were 245.43 (197.05–274.59) pg/mL in phakic and 359.27 (319.82–388.43) pg/mL in pseudophakic eyes.

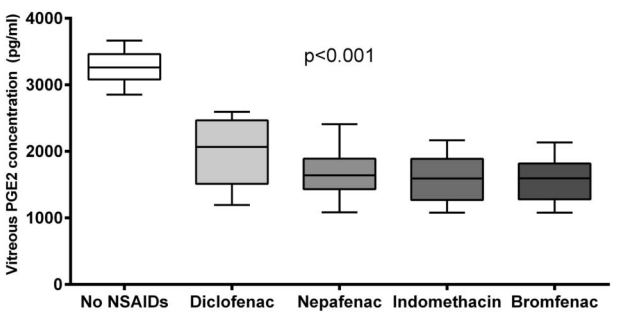

Fig. 1. Different vitreous PGE_2 levels for each study drugs. The decrease in vitreous PGE_2 levels was statistically significant for all the NSAIDs compared with the control group ($P < 0.001$).

Discussion

In the past few years, NSAIDs have been widely used in ophthalmology for the analgesic and anti-inflammatory activity. They play an important role in ophthalmic surgical procedures improving surgical outcomes and reducing postoperative inflammation and pain. In recent years, NSAIDs became a reasonable therapeutic approach to various vitreoretinal diseases.[8–13] Compared with aqueous drug levels, a paucity of scientific evidence measuring NSAIDs levels in human vitreous after topical administration was reported.

In our prospective and independent study, we quantitatively evaluated and compared the vitreous concentrations of diclofenac 0.1%, indomethacin 0.5%, nepafenac 0.3%, and bromfenac 0.09% and the related PGE_2 levels after topical administration. To the best of our knowledge, no published studies are reporting the vitreous concentration of diclofenac

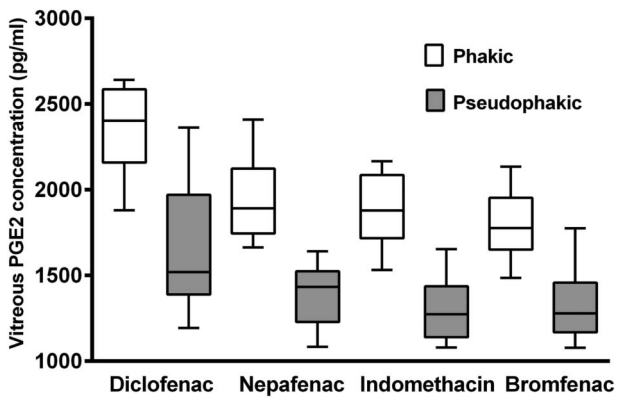

Fig. 2. Different vitreous PGE_2 levels according to the lens status. For each study group, a statistically significant difference was found between phakic and pseudophakic eyes ($P < 0.001$).

0.1% and nepafenac 0.3% in human eyes after topical administration and their effect on vitreous PGE_2 levels. In this study cohort of 104 eyes, we found a statistically significant difference in vitreous PGE_2 levels for all the NSAIDs groups compared with the control group ($P < 0.001$). In addition, a statistically significant difference between the diclofenac group and other assessed study drugs was detected ($P = 0.013$ for diclofenac vs. nepafenac; $P = 0.004$ for diclofenac vs. indomethacin or bromfenac). Similarly to previous studies, we found a statistically significant difference ($P < 0.001$) between phakic and pseudophakic patients both for NSAIDs and PGE_2 vitreous concentrations.

For many decades, NSAIDs have been used with a systemic administration. More recently, the topical ophthalmic formulations become available.[3] According to the past generation of NSAIDs treatment, several side effects from corneal stinging to corneal melt were found, and as result, their administration declined. In recent years, a new generation of NSAIDs with a quite safe profile for the ocular surface was developed.[2] Although the class of NSAIDs contains a chemically heterogeneous group of molecules, their topical use in ophthalmology is limited to soluble forms, including aryl acetic, indole acetic, and aryl propionic acid derivatives.[18,19]

The NSAIDs contrast the inflammation through the inhibition of COX enzymes, essential in the synthesis of prostanoids, particularly PGE_2 that is more commonly associated with inflammation. COX-1 is a constitutive isoform related to physiological functions and homeostatic activity. COX-2 is an inducible form associated with tissue trauma inflammation[20] and responsible for increased PGs activity. Both COX-1 and COX-2 enzymes are upregulated in response to inflammatory cytokines.[6]

Therefore, NSAIDs reduce the production of PGs, which can lead to the disruption of the blood–retinal barrier, vasodilatation, increasing vascular permeability, and leukocytosis. In the human retina, the COX-1 enzyme can be detected in retinal endothelial cells, ganglion cells, microglia, astrocytes, amacrine cells, Müller cells, and retinal pigment epithelium.[19] The COX-2 is the predominant isoform in the retinal pigment epithelium.[21]

In 2009, Heier et al[22] compared for the first time human vitreous PGE_2 levels after topical NSAIDs treatment. Patients were randomized to receive ketorolac 0.4% 4 times a day , bromfenac 0.09% twice a day, or nepafenac 0.1% 3 times a day, 3 days before surgery according to their recommended dosing regimen. In a control group of five patients, no NSAIDs were administered. The study demonstrated that all drugs penetrated into the vitreous cavity, and ketorolac-treated patients had significantly lower vitreous PGE_2 levels compared with the nepafenac ($P = 0.028$) and control group ($P = 0.047$). No statistically significant difference between the ketorolac and bromfenac groups was found. In this series of 31 eyes, a potential confounding factor could be the inclusion of different diseases between the NSAIDs and the placebo group. Four of five patients affected by retinal detachment were enrolled in the control group. This vitreoretinal condition leads to an increase in vitreous PGE_2 levels compared with ERM or MH. In addition, no information about phakic and pseudophakic eyes was provided, and no subgroup analysis according to the lens status was performed.

Schoenberger et al[23] demonstrated that topical application of ketorolac 0.45% four times daily 3 days before PPV achieved therapeutic vitreous levels sufficient to significantly decrease PGE_2 levels ($P = 0.04$). In this pilot study including 24 eyes, surgical indications were identical for both ketorolac and placebo groups, and according to lens status, a statistically significant higher vitreous concentration of ketorolac was found in pseudophakic eyes than in the phakic subgroup.

In a recent study by Russo et al,[24] 64 patients were randomized to receive topical indomethacin 0.5%, bromfenac 0.09%, nepafenac 0.1%, or placebo three times daily 7 days before surgery. The vitreous sample collected at the beginning of 25-gauge PPV for macular pucker revealed that all NSAIDs penetrated into the vitreous cavity, reaching a concentration sufficient to reduce vitreous PGE_2 levels ($P = 0.001$), without a significant difference among them ($P = 0.832$ for bromfenac vs. indomethacin; $P = 0.111$ for nepafenac vs. indomethacin). Significantly greater drug penetration in pseudophakic eyes compared with phakic eyes was observed.

A direct comparison between our results and those coming from previous studies evaluating NSAIDs and PGE_2 vitreous concentration was not possible. This is due to the different drug and vitreoretinal diseases included.

We found a lower difference in PGE_2 levels between the indomethacin and the bromfenac group than in the Russo et al[24] study. This difference could be related to the different dosage regimen and the period of treatment before the sample collection. In our series, the NSAIDs were administered 3 days before surgery and according to the own recommended dosing regimens. Our results are consistent with those previously reported by Bucolo et al[25] in an animal model analysis. The authors demonstrated that topical indomethacin, bromfenac, and nepafenac

significantly reduced retinal PGE_2 levels, with a higher effect of indomethacin and bromfenac compared with nepafenac. In a similar study by Kida et al,[20] the efficacy of bromfenac, diclofenac, and nepafenac topically administered in an animal model was assessed. The specific concentration for each NSAIDs was measured in the retinochoroidal tissue. In this experiment, a stronger inhibitory activity on COX-1 or COX-2 of bromfenac (from 2.7 to 10 times) than diclofenac or amfenac was found. These results reflect the difference in the pharmacokinetic profile for each NSAID. In particular, after the topical administration of the prodrug nepafenac, the intraocular hydrolases convert it to the more powerful active metabolite amfenac.

As demonstrated in an in vitro study, this hydroxylation results in a greater corneal permeability with an ocular penetration six times greater than diclofenac.[26] The chemical structure of bromfenac differs from that of amfenac by the presence of a bromine atom. This modification promotes a higher penetration of bromfenac in the ocular tissues and increases its anti-inflammatory activity.[21]

As reported in our study, each NSAID has different effectiveness in reducing posterior segment PGE_2 levels. Consistent with our results, a pilot study of 20 patients revealed that bromfenac is more effective than nepafenac in preventing PCME.[27]

Topical NSAIDs administration before cataract surgery has been regularly used as the standard of care since the mid-1980s. Mechanical damage related to surgical trauma causes a breakdown of the blood–aqueous and blood–retinal barrier promoting the inflammatory cascade and the release of PGE_2.[18–21,25] By countering prostanoids synthesis during cataract surgery, NSAIDs inhibit surgical-induced miosis, anterior chamber inflammation, and PCME and reduce perioperative ocular itching and pain.[5,28,29] In 2015, a report by the American Academy of Ophthalmology demonstrated the effectiveness of NSAIDs to reduce PCME compared with the placebo group or corticosteroid administration. However, the role of the long-term visual recovery after surgery remains unclear.[30]

In addition, a recent Cochrane analysis confirmed the prophylactic NSAIDs ability in reducing the risk of developing PCME, but according to the visual outcome, no statistical difference was found between the use of topical corticosteroid or topical NSAIDs.[31] Similar unclear effects on visual acuity were also reported in several meta-analyses.[4,30,32] Consequently, although these drugs reduce the incidence of PCME, it remains unclear whether the use of topical NSAIDs improves visual function and quality of life after cataract surgery.

Furthermore, NSAIDs administration had also been demonstrated to reduce postoperative pain and inflammation after vitreoretinal surgery when compared with placebo[33] and corticosteroids[15,34]

A growing body of scientific evidence suggests a role of PGs in several retinal diseases.[8–13] In several experimental systems, the inhibition of the COX enzyme can increase the efficacy of antiangiogenic treatments by suppressing vascular endothelial growth factor and angiogenesis.[35,36] According to the experimental models, human clinical trials reported the combination of topical NSAIDs and anti–vascular endothelial growth factor as a new synergetic strategy for the treatment of exudative age-related macular degeneration.[8]

Despite the relative paucity and controversial evidence about the use of topical NSAIDs in the management of uveitic cystoid macular edema,[37] many scientific reports support the role of NSAIDs in the treatment of diabetic macular edema[38–40] and macular edema secondary to branch retinal vein occlusion.[41.]

In this study, it has been observed that different NSAIDs determine a significant different reduction of PGE_2 vitreous concentration when compared with a placebo group. Even if vitreous concentrations of NSAIDs and PGE_2 are not a direct measure of retinal concentrations, this method of analysis, founded on a direct correlation between vitreous and retinal PGE_2 levels, is widely accepted. In addition, vitreous samples were collected at the beginning of the surgery, and therefore, the effects of NSAIDs were measured only on basal PGE_2 levels.

In summary, although diclofenac 0.1%, indomethacin 0.5%, nepafenac 0.3%, and bromfenac 0.09% achieve sufficient vitreous concentration to reduce basal vitreous PGE_2 levels, the PGE_2 concentration in the diclofenac group was significantly higher compared with the other NSAIDs. Moreover, indomethacin or bromfenac topical administration results in a greater reduction of PGE_2 levels compared with the nepafenac group. According to the lens status, a significantly higher drug bioavailability was found in pseudophakic than in phakic eyes, corresponding to a significantly lower PGE_2 concentration in all pseudophakic subgroups. To the best of our knowledge, this is the first report about the vitreous concentration of diclofenac 0.1% and nepafenac 0.3% after topical administration and their effect on vitreous PGE_2 levels in human eyes. For the first time, four different NSAIDs were directly compared in the same study. Additional independent clinical studies comparing topical NSAIDs effects would provide important insight into this topic and may strengthen these results about the therapeutic use of topical NSAIDs in the management of posterior segment inflammation.

Key words: full-thickness macular hole, idiopathic epiretinal membrane, nonsteroidal anti-inflammatory drugs, pars plana vitrectomy, prostaglandin E_2.

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