Inflammation, Vol. 24, No. 4, 2000

NEPAFENAC, A UNIQUE NONSTEROIDAL PRODRUG WITH POTENTIAL UTILITY IN THE TREATMENT OF TRAUMA-INDUCED OCULAR INFLAMMATION: I. Assessment of Anti-Inflammatory Efficacy

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Abstract—Nepafenac, the amide analog of 2-amino-3-benzoylbenzeneacetic acid (amfenac), was examined in preclinical models for its potential utility as a topical ocular anti-inflammatory agent. Diclofenac was selected as the reference compound. In contrast to diclofenac (IC_{50} = 0.12 μM), nepafenac exhibited only weak COX-1 inhibitory activity (IC_{50} = 64.3 μM). However, amfenac was a potent inhibitor of both COX-1 (IC_{50} = 0.25 μM) and COX-2 activity (IC_{50} = 0.15 μM). Ex vivo, a single topical ocular dose of nepafenac (0.1%) inhibited prostaglandin synthesis in the iris/ciliary body (85–95%) and the retina/choroid (55%). These levels of inhibition were sustained for 6 h in the iris/ciliary body and 4 h in the retina/choroid. Diclofenac (0.1%) suppressed iris/ciliary body prostaglandin synthesis (100%) for only 20 min, with 75% recovery observed within 6 h following topical dosing. Diclofenac's inhibition of prostaglandin synthesis in the retina/choroid was minimal. Nepafenac's inhibitory efficacy and longer duration of action was confirmed in a trauma-induced rabbit model of acute ocular inflammation monitoring protein or PGE_2 accumulation in aqueous humor. Results warrant further assessment of nepafenac's topical ocular efficacy in the treatment of postoperative ocular pain, inflammation, and posterior segment edema.

INTRODUCTION

Amfenac (2-amino-3-benzoylbenzeneacetic acid) is a non-steroidal anti-inflammatory compound with an arylacetic acid structure which exhibits potent antipyretic and analgesic properties (1). The anti-inflammatory and analgesic properties of this molecule are mediated through inhibition of cyclooxygenase (prostaglandin H synthase) activity (2, 3). The potent analgesic properties estab-

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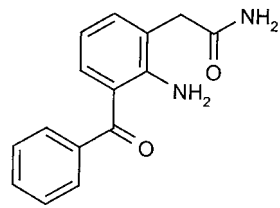

Fig. 1. Nepafenac, 2-amino-3-benzoylbenzeneacetamide.

lished in preclinical models have been confirmed in a double-blind, randomized clinical trial evaluating amfenac's efficacy in suppressing post-surgical pain following extraction of impacted molar teeth (4). Amfenac sodium has been approved in Japan and is marketed as Fenazox® for the treatment of rheumatoid arthritis and for pain and/or inflammation after surgery (5). A series of structural analogues of amfenac have been prepared to enhance the compound's therapeutic index (3). In these studies it was noted that the amide analog of 2-amino-3-benzoylbenzeneacetic acid serves as a prodrug exhibiting potent anti-inflammatory activity but greatly reduced intestinal toxicity.

Despite a plethora of prostaglandin synthase inhibitors available for systemic use, there is a continued need for topical ocular prostaglandin H synthase inhibitors that are comfortable and efficacious in suppressing postoperative pain, inflammation and development of cystoid macular edema (6).

Nepafenac (Figure 1) is a potent, systemic nonsteroidal antiinflammatory prodrug (3). In the present report we examine nepafenac for its a) intrinsic prostaglandin H synthase inhibitory activity, b) ex vivo inhibition of prostaglandin synthesis in ocular tissue, and c) anti-inflammatory activity in a trauma (paracentesis)-induced model of acute ocular inflammation in the NZA rabbit following a single topical ocular dose. These studies were designed to explore the compound's potential as a topical ocular anti-inflammatory agent. The results are contrasted to those obtained with diclofenac, marketed as Voltaren® for the treatment of post-surgical ocular inflammation and suppression of ocular pain following photorefractive keratotomy.

MATERIALS AND METHODS

Animals. New Zealand Albino rabbits (2–2.5 kg) were obtained from Myrtle's Rabbitry, Thompson Station, Tennessee. All aspects of handling, housing and experimentation conformed with the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research.

Materials. Nepafenac was synthesized by the medicinal chemistry unit, Alcon Laboratories, Inc. (Fort Worth, Texas). Diclofenac sodium was purchased from Sigma Chemical Co. (St. Louis,

Missouri). For topical ocular administration, the compounds were prepared in an ophthalmic vehicle containing 0.5% hydroxypropyl methylcellulose. Alternatively, a commercial preparation of diclofenac 0.1% (Voltaren® Ophthalmic, CibaVision Ophthalmics, Atlanta, Georgia) was employed, as indicated in the Results section. Alcaine® Ophthalmic Solution (0.5% proparacaine hydrochloride) is also a product of Alcon Labs. All concentrations are expressed on a weight/volume basis.

Other reagents were obtained as follows: eicosatetraenoic (arachidonic) acid, NuCheck Prep (Elysian, Minnesota); [1-^{14}C]-eicosatetraenoic acid (53 μCi/μmol) and PGE_2 [^{125}I]-RIA kits, both from NEN (Boston, Massachusetts); NS-398 and PGH synthase-2 (COX-2) from sheep placenta (6500 units/mg), both from Cayman Chemical Company (Ann Arbor, Maine). All other chemicals were products from either Sigma Chemical Company (St. Louis, Missouri) or J. T. Baker (Phillipsburg, New Jersey). Solvents used for tissue extraction and HPLC analysis were either of HPLC grade or the highest purity available (EM Science, Gibbstown, New Jersey). Coomassie protein assay reagent was obtained from Pierce Chemical (Rockford, Illinois). Budget Solve (liquid scintillant) was a product of RPI (Mount Prospect, Illinois).

PGH Synthase Activity in Vitro. Inhibition of PGH synthase activity from sheep vesicular glands (COX-1) was assayed polarographically (7) with a YSI model 53 oxygen monitor as previously described (8). Briefly, 10 μl of solubilized microsomal preparation (20 mg/ml) was added to the YSI incubation chamber containing 3.0 mL of 50 mM phosphate buffer (pH 7.4), 0.5 mM phenol, and 0.5 μM hematin. Aliquots (≤50 μL) of carrier or test compound dissolved in DMSO (carrier) were added to the chamber which was pre-equilibrated to 30°C. The mixture was stirred for two minutes before the reaction was initiated by addition of 30 μL of ammonium arachidonate (10 mM).

PGH synthase activity from sheep placenta (COX-2) was assayed exactly as described above with the exception that the amount of enzyme added to the reaction chamber was reduced to 20 μg protein.

PG Synthesis of Tissue Homogenates of the Iris/Ciliary Body and the Retina/Choroid. PG synthesis was evaluated in homogenates of iris/ciliary body and retina/choroid at various times ranging from 5 min through 8 h after bilateral administration of a topical ocular dose (50 μL) of either test drug or vehicle. Iris/ciliary body tissue or retina/choroid tissue was placed in a polypropylene tube containing 0.5 mL of ice-cold, 50 mM phosphate/0.5 mM phenol buffer (pH 7.4) and homogenized with a Brinkman homogenizer. The homogenate preparation, incubation and extraction procedures were performed as previously detailed (9).

To monitor eicosanoid formation by radio-HPLC, homogenates were incubated for 10 min at 37°C with 10 μM of [1-^{14}C]-labeled arachidonic acid (10 μCi/μmol). The extraction procedure employed yielded an overall isotope recovery of 78.9 ± 5.9% (mean ± SD) when [1-^{14}C]-arachidonic acid was used as substrate. PGE_2 was quantified in organic extracts by HPLC as described by Powell (10). Alternatively, synthesis of PGE_2 was assessed by [^{125}I]-labeled RIA following a 10 min incubation of tissue homogenates of the iris/ciliary body and retina/choroid with unlabeled arachidonic acid (10 μM). The incubation conditions and method of extraction of arachidonic acid metabolites were identical to those described above for the radiometric/HPLC assay procedure.

Protein concentration of tissue homogenates was assayed according to the colorimetric method described by Bradford (11).

Trauma-Induced Breakdown of the Blood-Aqueous Barrier. The effect of prophylactic topical ocular administration of a single dose of either nepafenac, diclofenac or vehicle on paracentesis-induced breakdown of the blood-aqueous barrier was assessed in the NZA rabbit. The procedure has been previously described in detail (9). Briefly, animals received a single topical ocular dose of test compound or vehicle (50 μL), administered bilaterally. Forty-five min after dosing, 1 drop (5 μL) of 0.5% proparacaine was instilled in each eye, and trauma was elicited by paracentesis within 5 min. (All modifications of the pretreatment interval are noted in the Results section.) Removal of aqueous humor (≈150 μl per eye) was accomplished by puncture of the cornea with a 27 g needle. Of

this volume, 100 μl was diluted with an equal volume of a 2% solution of EDTA in saline (pH 7.4), frozen on dry ice and stored at −70°C for later analysis of protein and PGE_2 content. Protein and PGE_2 levels in the aqueous humor samples obtained before and after paracentesis were determined as noted above.

Thirty min following the initial paracentesis, animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg) via the marginal ear vein. Post-trauma aqueous humor samples were obtained immediately, and were stored and analyzed as described above.

RESULTS

Inhibition of PGH Synthase. In vitro studies were conducted to evaluate the inhibitory potency of nepafenac in suppressing the activity of a partially purified preparation of PGH synthase-1 from sheep vesicular glands. The free acid analog amfenac and diclofenac served as reference compounds. Nepafenac, amfenac and diclofenac each caused a concentration dependent inhibition of PGH synthase activity as measured polarographically by decreased rates in oxygen consumption. All compounds produced comparable maximal inhibition (83%–88%) at their highest test concentrations (Table 1). Nepafenac clearly exhibited the lowest PGH synthase-1 inhibitory potency, with an IC_{50} of 64.3 μM. Diclofenac and the free carboxylic acid analog, amfenac, were about 500- and 250-fold more potent than nepafenac, with IC_{50} values of 0.12 μM and 0.25 μM, respectively. As an inhibitor of PGH synthase-2 activity, amfenac produced half-maximal inhibition at a concentration of 0.15 μM. The reference compound NS-398 was a less potent inhibitor of this enzyme activity than amfenac, with an IC_{50} value of 0.26 μM.

Table 1. In Vitro Inhibition of Prostaglandin H Synthase

Test compound	Concentration test range [μM]	IC_{50} [μM]	Correlation coefficient (r-value) (n)[a]	Inhibition (%) at highest test concentration
Prostaglandin H Synthase-1[b]				
Nepafenac	3.9–260	64.3	0.99 (6)	83.0
Amfenac	0.12–0.60	0.25	0.92 (5)	84.4
Diclofenac	0.022–0.37	0.12	0.95 (12)	88.2
Prostaglandin H Synthase-2[c]				
NS-398	0.053–1.6	0.26	0.88 (11)	87.9
Amfenac	0.012–1.2	0.15	0.86 (8)	87.5

[a]Number of data points.
[b]Vesicular gland enzyme (COX-1).
[c]Placenta enzyme (COX-2).

Table 2. ExVivo Inhibition of Prostaglandin Synthesis of the Iris/Ciliary Body in the NZA Rabbit 60 Min Following a Single Topical Ocular Dose of Diclofenac and Nepafenac

		[% Inhibition]	
Arachidonic acid metabolite	Naive control [nmol/10 min/100 mg tissue] (N = 32)	Diclofenac [0.1%] (N = 7)	Nepafenac [0.1%] (N = 16)
PGI_2	0.64 ± 0.21	42.1 ± 27.6[a,b]	59.9 ± 13.2[a]
TXB_2	0.39 ± 0.28	51.2 ± 26[a,b,c]	78.3 ± 30.7[a]
$PGF_{2\alpha}$	0.27 ± 0.06		96.7 ± 9.0[a]
PGE_2	0.63 ± 0.25	65.7 ± 17.6[a,b,d]	90.5 ± 13.0[a]
PGD_2	0.13 ± 0.14		100 ± 0[a]
HHT	0.96 ± 0.35	43.1 ± 29.8[a,b]	63.0 ± 8.7[a]

[a]Significantly different from naive control ($P < 0.05$; Welch test).
[b]Significantly different from nepafenac ($P < 0.05$; Welch test).
[c]Expressed as combined inhibition of $TXB_2/PGF_{2\alpha}$ due to lack of resolution by radio-HPLC.
[d]Expressed as combined inhibition of PGE_2/PGD_2 due to lack of resolution by radio-HPLC.

Ex Vivo PG Synthesis. To obtain direct evidence of suppression of PGH synthase activity in ocular tissue, ex vivo studies were conducted monitoring PG formation in the iris/ciliary body following a single topical ocular administration of nepafenac (0.1%). Parallel studies employed the reference compound diclofenac (0.1%).

A single topical ocular administration of diclofenac (50 μL, 0.1%) significantly inhibited all prostanoid production by the iris/ciliary body excised 60 minutes following dosing (Table 2). Inhibition ranged from 42% to 65%, with PGE_2/PGD_2 synthesis being most suppressed. Notably, nepafenac (50 μL, 0.1%) promoted significantly greater inhibition. PGE_2, $PGF_{2\alpha}$, and PGD_2 synthesis was nearly abolished, while PGI_2 (measured as 6-keto-$PGF_{1\alpha}$), and TXB_2/HHT production was reduced by 2/3 compared to that produced by tissue from naive animals.

The greater suppression (≥95%) of PG synthesis in the ICB by a single topical ocular administration of nepafenac was more closely examined using time points of 5, 10, 20, 40 and 80 min post dosing. Total iris/ciliary body PG production rapidly declined in both nepafenac treated and diclofenac treated animals (Figure 2). At the earliest time point examined (5 min) diclofenac (0.1%) and nepafenac (0.1%) reduced total PG synthesis by 41% and 58%, respectively. The inhibition of PG synthesis following diclofenac administration increased little thereafter, with peak inhibition (53%) observed 80 min after dosing. In contrast, animals receiving a single dose of 0.1% nepafenac exhibited progressively greater inhibition of total PG synthesis by the iris/ciliary body with time. Greater than 95% inhibition was achieved 80 min following administration. Nepafenac

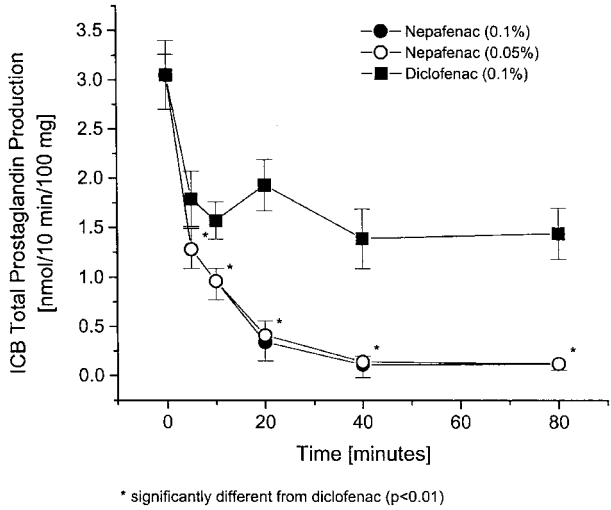

Fig. 2. Ex vivo inhibition of prostaglandin synthesis in the iris/ciliary body of the NZA rabbit following a single topical ocular administration of nepafenac (0.05% and 0.1%) and diclofenac (0.1%). [1-^{14}C]-eicosanoid formation was determined by quantitative radio-HPLC analysis. Each data point represents the mean ± *SD* (*N* = 6).

was equally efficacious when employed at a concentration of 0.05%. Although diclofenac exhibits greater intrinsic CO activity than the active metabolite of nepafenac (Table 1), nepafenac is a more efficacious in vivo inhibitor of PG synthesis when applied topically to the eye.

The duration of PGE_2 synthesis inhibition in the iris/ciliary body was then monitored through 6 h following a single topical ocular dose of nepafenac or diclofenac (Figure 3). In this study PGE_2 formation was determined by RIA rather than by [1–^{14}C]-eicosanoid formation by radio-HPLC. Diclofenac (0.1%) abolished iris/ciliary body synthesis of PGE_2. This level of inhibition was sustained for ≈20 min following drug administration. Thereafter, inhibition of PGE_2 synthesis declined steadily to 26% six h after dosing. In contrast, nepafenac (0.1%) exhibited a somewhat slower onset (20 min) to maximal suppression (90%–95%) of PGE_2 synthesis. However, this level of suppression was sustained for at least 6 h, demonstrating nepafenac's greater duration of action.

In the retina/choroid (Figure 4) inhibition of PGE_2 synthesis was observed earlier in nepafenac treated animals (40 min) than in diclofenac treated animals (80 min) following a single topical ocular dose (0.1%). Whereas the iris/ciliary body showed nearly complete inhibition of PGE_2 synthesis immediately following nepafenac treatment, in the retina/choroid PGE_2 synthesis was partially suppressed (55%) at 40 min following dosing. This level of inhibition was sustained

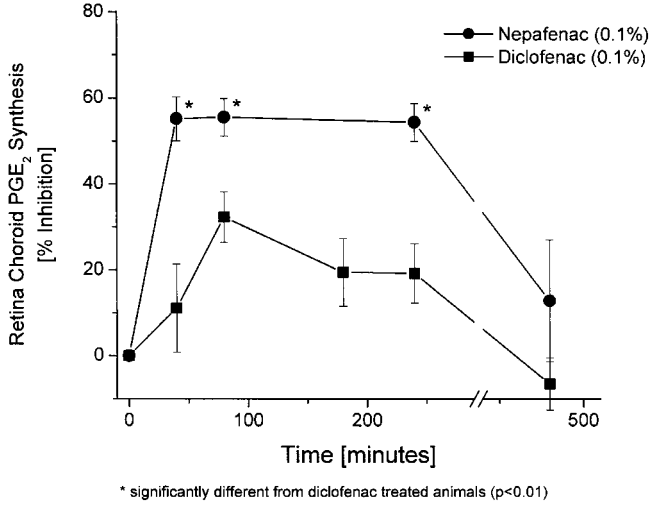

Fig. 3. Ex vivo inhibition of PGE_2 synthesis in the iris/ciliary body of the NZA rabbit after various times following a single topical ocular administration of nepafenac (0.1%) and diclofenac (0.1%). PGE_2 production was assessed by RIA. The rate of PGE_2 production of tissue homogenates from the iris/ciliary body of untreated (control) animals was 202 ± 74 ng/10 min/mg protein (N = 32). Each data point represents the mean ± SD (N = 8).

for a period of 4 h. Thereafter PGE_2 inhibition declined to 13% eight hours after dosing. Diclofenac (0.1%) treatment produced only 20–32% inhibition of PGE_2 synthesis. This level of inhibition was observed 80 min through 4 h following drug administration. Eight h post diclofenac dosing there was no inhibition of PGE_2 synthesis by retina/choroid.

Inhibition of Blood Aqueous Barrier Breakdown. To evaluate the anti-inflammatory activity of topical ocular nepafenac animals were dosed 45 min prior to paracentesis with test compound or vehicle. Aqueous humor protein and PGE_2 concentrations were assessed thirty min following paracentesis.

A single, prophylactic topical ocular dose of nepafenac (0.1%) significantly reduced paracentesis-induced accumulation of protein (61% reduction) and nearly abolished PGE_2 accumulation (98% reduction) in the aqueous humor (Figure 5). This inhibitory effect was very similar to that observed with 0.1% diclofenac. Amfenac (0.1%) inhibited aqueous humor protein accumulation by 59%.

The onset and duration of action of a single 0.1% dose of nepafenac was compared to that of diclofenac 0.1% (Voltaren). Rabbits were dosed topically, and trauma was subsequently induced at various times. Nepafenac maintained significant anti-inflammatory efficacy from 15 min through eight hours following a single

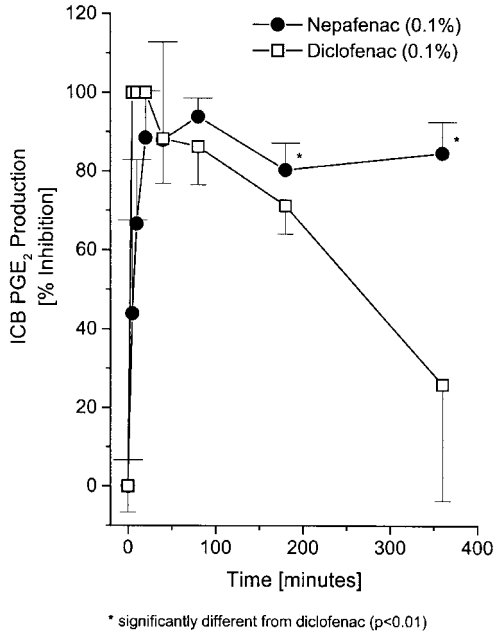

Fig. 4. Ex vivo inhibition of PGE_2 synthesis of the retina/choroid of the NZA rabbit after various times of a single topical ocular administration of nepafenac (0.1%) and diclofenac (0.1%). Experimental details and assessment of PGE_2 production by RIA are described in Methods. The rate of PGE_2 production of tissue homogenates from the retina/choroid of untreated (control) animals was 55 ± 5 ng/10 min/mg protein (N = 26). Each data point represents the mean ± *SEM* ($N \geq 8$).

topical dose (Figure 6). Diclofenac inhibited the vascular permeability response 15 min through 4 h post-dosing, but failed to do so at the 8 h time point.

Nepafenac's inhibition of protein extravasation in the anterior chamber of the eye was shown to be concentration dependent (Figure 7). Significant anti-inflammatory activity was observed at concentrations as low as 0.01%. Maximum efficacy in this model was noted at 0.03% and was maintained at doses up to 0.3%. This maximum efficacy was comparable to that observed with diclofenac 0.1%.

DISCUSSION

Classical nonsteroidal anti-inflammatory drugs with arylacetic acid structure are potent inhibitors of PGH synthase that promote a time-dependent inactivation of the enzyme with increasing time of drug exposure (12). This inhibitory

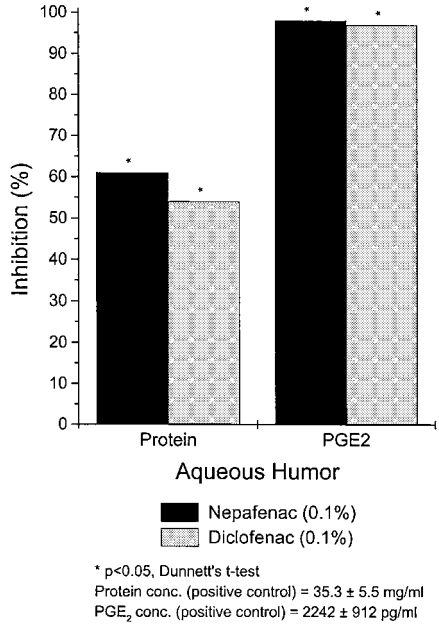

Fig. 5. Inhibition by topical ocular nepafenac of protein and PGE_2 accumulation in aqueous humor following paracentesis in NZA rabbits (N = 8 eyes/treatment group).

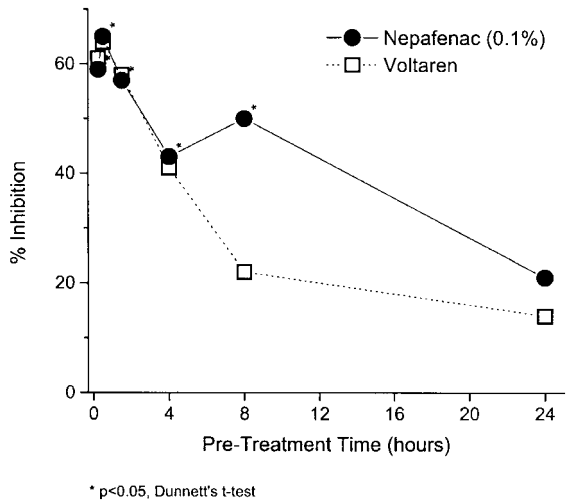

Fig. 6. Duration of action of nepafenac 0.1% on paracentesis-induced blood-aqueous-barrier breakdown in NZA rabbits following topical ocular administration (N = 8 eyes/ treatment group).

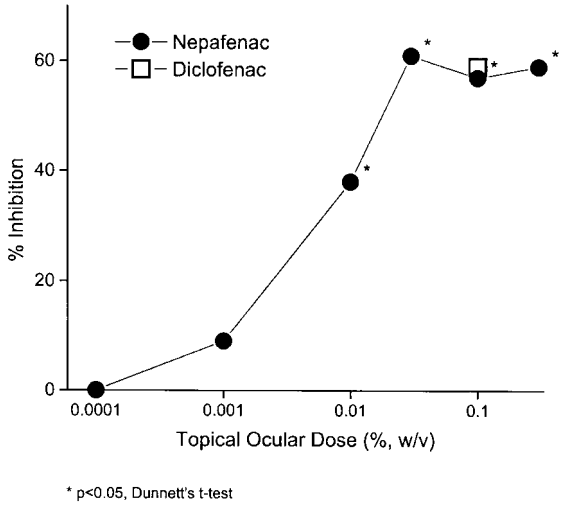

Fig. 7. Concentration dependent effect of topically applied nepafenac on blood-aqueous barrier breakdown in NZA rabbits following paracentesis ($N \geq 8$ eyes/treatment group).

characteristic is abolished with chemical modification of the carboxyl functional group (i.e., esterification) (12). The latter behavior is demonstrated in the present study for nepafenac, representing the amide analog of amfenac. As an amide analog, nepafenac exhibits weak intrinsic PGH synthase-1 inhibitory potency (IC_{50} = 64.3 μM), whereas its free carboxylic acid counterpart, amfenac, causes potent inhibition of both PGH synthase-1 (COX-1) (IC_{50} = 0.25 μM) and PGH synthase-2 (COX-2) (IC_{50} = 0.15 μM). This potency in suppressing COX-2 suggests that nepafenac may have advantages over non-COX-2 inhibitory NSAIDs in treating existing ocular inflammatory conditions.

When tested for anti-inflammatory activity in a trauma-induced (paracentesis) rabbit model of ocular inflammation, topical prophylactic administration of nepafenac (0.1%) yielded efficacy equal to that of the arylacetic acids, amfenac (0.1%) and diclofenac (0.1%), the reference compound, in suppressing both PGE_2 (>95%) and protein accumulation (61%) in the aqueous humor. This observation suggested that nepafenac, which exhibits only weak intrinsic COX inhibitory activity, readily undergoes bioactivation to amfenac by intraocular hydrolases following penetration of external ocular barriers. Results obtained from the ex vivo experiments described confirmed the bioavailability of an active cyclooxygenase inhibitor following topical dosing. Not only was PG synthesis reduced in iris/ciliary body tissue removed from animals dosed topically with nepafenac, but significant reduction in PG production was observed in retina/choroid removed following topical dosing.

Superior pharmacodynamics were apparent for nepafenac compared to diclofenac in these studies. This was evident by both a more efficient suppression of all PG produced by the iris/ciliary body (Table 2 and Figure 5) and a longer duration of PG synthesis suppression (Figure 6). The relatively fast recovery of PG synthesis of the iris/ciliary body observed with diclofenac treatment further indicates a relatively short half-life of diclofenac in tissues of the anterior chamber. This is indicated by a 70% recovery in PG synthetic capacity of the iris/ciliary body 6 h following a single topical ocular dose of diclofenac. Based on an estimated 7.7 h time interval for half-maximal recovery of iris/ciliary body synthesis of PGH following self-catalyzed inactivation upon paracentesis (9), there seems to be insufficient diclofenac available in the anterior chamber and/or iris/ciliary body to inhibit newly expressed enzyme, even as early as 40 min after topical dosing (Figure 6). This is clearly in contrast to nepafenac, which exhibited sustained inhibition of PG synthesis through 6 h following a single topical ocular dose. An assessment of the in vitro conversion of nepafenac to amfenac in corneal tissue slices, iris/ciliary body, and retina/choroid isolated from NZA rabbits and from human cadaver eyes confirms the bioconversion of nepafenac to amfenac (13).

Most interesting is the observation that a single topical ocular dose of nepafenac (0.1%) caused a significant suppression in PGE_2 synthesis (55%) of the retina/choroid of the NZA rabbit. This level of suppression was sustained for a period of 4 h prior to returning to baseline levels. These results indicate that topical ocular nepafenac is readily bioavailable to the retina/choroid, likely through both corneal and scleral absorption. Following absorption, hydrolytic conversion to amfenac is readily accomplished by the retina/choroid which exhibits the highest ocular levels of hydrolase activity (6.5 nM/min/mg). Diclofenac, on the other hand, promoted only a marginal suppression (20%–32%) of PGE_2 synthesis over the same time course, indicating lower retinal bioavailability.

Previously published studies have demonstrated that trauma elicited immediate and transient PGE_2 synthesis in the anterior chamber of the eye associated with breakdown of the blood-aqueous barrier (9). Aqueous PGE_2 levels were significantly elevated within 10 min post paracentesis, but decreased steadily beyond 20 min. Following a brief delay, blood-aqueous barrier breakdown, as indicated by the accumulation of vascular derived protein in aqueous humor samples, peaked by 30 min, with a gradual decline thereafter. The characteristics of this ocular inflammatory response suggested that studies evaluating topical ocular efficacy of PG synthesis inhibitors should employ a drug pre-treatment regimen (9). Therefore, in the present studies, animals were pretreated with a single topical ocular administration 45 min prior to injury. Effects of the drugs on aqueous humor protein and PGE_2 concentrations were compared at the peak of the inflammatory response, 30 min following paracentesis.

Nepafenac produced concentration dependent inhibition of trauma-induced inflammation following a single topical ocular administration. Significant anti-inflammatory efficacy was observed at concentrations as low as 0.01%. While nepafenac 0.1% and Voltaren were indistinguishable in efficacy, nepafenac exhibited superior pharmacodynamic properties in these rabbit studies. Nepafenac maintained significant activity through 8 h, while Voltaren was inactive by this time. These results demonstrate a superior duration of action for nepafenac compared with Voltaren. Also, this investigation suggests that the drugs display a comparable onset of action despite the topical application of a prodrug in the case of nepafenac. This early onset of action of the amide prodrug is consistent with rapid corneal penetration and subsequent conversion to the free acid in the iris/ciliary body and retina/choroid, as described by Graff et al (13). Rapid corneal penetration favors the accumulation of adequate nepafenac concentrations at the iris-ciliary body, which comprises the blood-aqueous barrier and a site of significant PG synthesis (14).

The results obtained from investigations of the intrinsic PGH synthase activity and inhibition of PG synthesis in ocular tissue of the rabbit following a single topical ocular dose, and efficacy to inhibit trauma-induced ocular inflammation in the rabbit, demonstrate that nepafenac exhibits ocular anti-inflammatory properties superior to diclofenac. Although nepafenac requires intraocular bioactivation to become an effective PGH synthase inhibitor, its superior penetration of both the cornea and scleral tissue allows for an apparently greater intraocular drug accumulation than is achieved with diclofenac. The favorable ocular penetration of nepafenac and its rapid intraocular metabolism to amfenac at target sites readily overcomes the two-fold greater PGH synthase inhibitor potency advantage of diclofenac, thus providing equal or greater anti-inflammatory efficacy and greater duration of PG synthesis suppression in preclinical animal models than observed with diclofenac. Importantly, clinical evaluation of nepafenac in post cataract surgery inflammation demonstrated significant anti-inflammatory efficacy (15).

In addition to suppressing inflammation in the anterior chamber, results suggest that nepafenac may be employed in conditions involving the posterior segment of the eye. Cystoid macular edema is the most frequent cause of vision loss following cataract extraction (16). Characterized by cystic swelling of the macula associated with vascular leakage as detected by fluorescein angiography (17), this condition is evident postoperatively in 50 to 70% of cataract patients. The observation that cyclooxygenase inhibitors can attenuate post-surgical macular edema (18, 19) warrants an efficacy assessment of nepafenac, which is readily bioavailable to the posterior segment following topical ocular administration. Suppression of PG synthesis may attenuate not only the post-surgical edematous response but also the elevation of vasodilatory PG observed preclinically in the retinal vasculature under diabetic conditions (20). A key finding in

the present studies is the demonstration that topical application of nepafenac produced significant inhibition of PG synthesis by retinal and choroidal tissue ex vivo. Diclofenac (0.1%) was inactive in posterior ocular tissues when tested under identical conditions. Therefore, the preclinical pharmacodynamic profile exhibited by nepafenac indicates the potential to suppress PG-mediated inflammation in both anterior segment tissues and retinal tissue following topical ocular administration.

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