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Inflammation-Mediated Retinal Edema in the Rabbit Is Inhibited by Topical Nepafenac

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Abstract—The purpose of this study was to evaluate the ability of the nonsteroidal anti-inflammatory drug nepafenac to prevent development of mitogen-induced pan-retinal edema following topical ocular application in the rabbit. Anesthetized Dutch Belted rabbits were injected intravitreally (30 μg/20 μL) with the mitogen concanavalin A to induce posterior segment inflammation and thickening (edema) of the retina. The Heidelberg Retina Tomograph was used to generate edema maps using custom software. Blood-retinal barrier breakdown was assessed by determining the protein concentration in vitreous humor, whereas analysis of PGE_2 in vitreous humor was performed by radioimmunoassay. Inhibition of concanavalin A-induced retinal edema was assessed 72 h after initiation of topical treatment with nepafenac (0.1–1.0%, w/v), dexamethasone (0.1%), VOLTAREN® (0.1%), or ACULAR® (0.5%). Concanavalin A elicited marked increases in vitreal protein and PGE_2 synthesis at 72 h postinjection. Retinal thickness was also increased by 32%, concomitant with the inflammatory response. Topical application of 0.5% nepafenac produced 65% reduction in retinal edema which was correlated with 62% inhibition of blood-retinal barrier breakdown. In a subsequent study, 0.5% nepafenac significantly inhibited (46%) blood-retinal barrier breakdown concomitant with near total suppression of PGE_2 synthesis (96%). Neither Voltaren nor Acular inhibited accumulation of these markers of inflammation in the vitreous when tested in parallel. This study demonstrates that nepafenac exhibits superior pharmacodynamic properties in the posterior segment following topical ocular dosing, suggesting a unique therapeutic potential for a variety of conditions associated with retinal edema.

KEY WORDS: nepafenac; inflammation; retinal edema; NSAID.

INTRODUCTION

Retinal edema is a major cause of visual dysfunction associated with disorders such as diabetic retinopathy, vein occlusion, postsurgical complications, and trauma. In diabetes patients 20–74 years of age, diabetic retinopathy is the most frequent cause of new cases of blindness (1). Diabetic retinopathy affects over 5.3 million Americans aged 18 or older, or just over 2.5% of this population (2). In the preproliferative (background) form, the major cause of visual dysfunction is macular edema. Macular edema will ultimately develop in 42% of type I diabetics (insulin-dependent diabetic mellitus) and more than 80% of type II diabetics (non-insulin-dependent diabetic mellitus) after 15 years of diabetes (3). As inflammation and cell signaling have been implicated in the generation of edema (diffuse and cystoid), evidence has accumulated to support the use of anti-inflammatory therapy for stabilizing or improving vision associated with various retinal disorders such as diabetic retinopathy.

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Nepafenac is a prodrug of a nonsteroidal anti-inflammatory drug (NSAID) that functions by inhibiting cyclooxygenase enzymes and, thereby, the synthesis of endogenous pro-inflammatory prostaglandins. In animal studies, prostaglandins have been shown to produce disruption of the blood-aqueous barrier, promote vasodilation, and increase vascular permeability—changes similar to those seen in retinal edema. The presumed mode of action is consistent with the expected benefits of use of this agent. Nepafenac is a weak *in vitro* inhibitor of cyclooxygenase activity ($IC_{50} = 64\ \mu M$) and must be metabolized *in vivo* to demonstrate potent cyclooxygenase inhibitory activity (4). Significantly, nepafenac rapidly penetrates the cornea and is converted by amidases, found primarily in iris/ciliary body (ICB) and retina/choroid, to the free arylacetic acid analog, amfenac (5). This metabolic conversion occurs quickly. Within 15 min, nepafenac significantly inhibits prostaglandin synthesis in the ICB *ex vivo*, and exhibits a maximum inhibitory effect *in vivo* on paracentesis-induced vascular permeability and PGE_2 accumulation in aqueous humor (60%), similar to that observed with the reference nonsteroidal anti-inflammatory, diclofenac. The purpose of these studies was to evaluate the ability of nepafenac, administered topically, to prevent the development of inflammation-mediated pan-retinal edema. Severe, pan-retinal inflammation and degeneration were induced in the pigmented rabbit retina following the intravitreal injection of the mitogen concanavalin A. Edema and posterior segment inflammation were assessed by measuring changes in retinal thickness, retina PGE_2 synthesis, and protein leakage in the vitreous humor.

METHODS

Animals

All aspects of animal handling, housing, and experimentation conformed to the ARVO Resolution for the use of animals in ophthalmic and vision research. Dutch Belted Rabbits of either sex, weighing 1.7–2.4 kg were used in the conduct of pharmacology studies. New Zealandwhite (NZW) rabbits weighing 2.1–2.3 kg were used to examine the ocular distribution of nepafenac. Prior to and during the experimental period, the rabbits were maintained in a controlled environment with a 12-h on/off light cycle at $65 \pm 5^{\circ}F$ and at least 30% humidity. Food and water were administered *ad libitum*.

Pan-Retinal Inflammation

Induction of pan-retinal inflammation in the Dutch Belted rabbit was previously described by Kapin *et al.* (6) Briefly, the procedure entailed the intravitreal injection of concanavalin A (30 μg/20 μL sterile saline) in the anesthetized (ketamine 45 mg/kg/xylazine 5 mg/kg; sc) rabbit. For evaluation of efficacy following topical ocular administration, nepafenac (1% (0.3 μg/drop), 0.5% (0.15 μg/drop), 0.01% (0.03 μg/drop)) or vehicle ($\sim$30 μL by Droptainer™) was instilled five times per day beginning 1 day prior to intravitreal injection of concanavalin A and continuing until termination of the in-life phase of the study, 72 h after concanavalin A injection. To assess activity following systemic administration, animals received a single daily subcutaneous injection of drug or vehicle on days -1, 0, 1, 2, and 3 relative to concanavalin A injection. Final retinal images were acquired 72 h post-concanavalin A injection.

Tissue Collection and Biochemical Analysis of Ocular Tissues and Fluids

At various times following concanavalin A injection, the rabbits were euthanized by intravenous administration of sodium pentobarbital (SLEEPAWAY® Fort Dodge, IA). Following confirmation of death, eyes were proptosed and 100 μL of aqueous humor collected with a 25-g needle, mixed with an equal volume of 2% EDTA (pH 7.0) and frozen on dry ice. The eyes were then enucleated and placed in ice cold PBS (Sigma). The globe was dissected on ice to collect tissue from the posterior segment. A circumflex cut was made 1–3 mm behind the limbus and the vitreous body was separated from the anterior segment by gently pulling the sclera. The vitreous body was cut free from the anterior segment, placed in 50 μL of a protease inhibitor cocktail (5 mM EDTA, 0.5 mM AEBSF, 5 mM Benzamadine, 1 mM pepstatin, and 1 mM leupeptin) and frozen on dry ice. All samples were stored at $-80^{\circ}C$ pending analysis.

Assessement of Vascular Leakage

Breakdown of the blood-retinal barrier was assessed by determining protein concentration in the vitreous compartment. Protein analysis was performed using a modification of the method of Bradford *et al.* (7) Coomassie Protein Assay Reagent (Pierce Chemical Co.) was used for quantification of standards and samples. BSA (2 mg/mL, Pierce) was used as the standard for the assay. The detection range for the standard curve was 2–20 μg of BSA. To 20 μL of sample contained in 12×75 mm glass tubes, 1 mL of Pierce Assay Reagent was added and mixed. The optical density of the standards and samples was measured

at 595 nm using a DU®-65 spectrophotometer (Beckman Coulter). The protein concentration in the samples was calculated from a linear regression of the standard curve and corrected for any dilution.

Analysis of Retinal Thickness

Analysis of retinal thickness changes was performed using the Heidelberg Retina Tomograph and software package version 2.01 b-MS. This method is illustrated in Fig. 1 and described in detail elsewhere (8). Briefly, the procedure used autoregistry landmarks followed by repetitive laser scans. For autoregistry purposes, an argon laser was used to place three landmark scars (100 mW, 150-μm spot width, 0.1-ms duration) in a 10° radius, 1 disc diameter inferior to the optic nerve head of anesthetized study rabbits (Fig. 2). After a 2-week recovery period, baseline retinal thickness measurements (7 images/animal) were determined in the anesthetized rabbits. An additional baseline retinal thickness determination (7 images/animal) was conducted prior to intravitreal injection of 30-μg concanvalin A. Final images were obtained at 72-h postinjection.

The relative mean retinal thickness was quantified for each image by analyzing the distribution of reflectance intensity as a function of scan depth (*z*-profile) weighted by the peak reflectance intensity at each pixel. The resultant edema map gave an edema index (EI) for each pixel and the mean EI (MEI) was calculated for the entire image or any predefined subregion. Custom software (Tview) was used to align all images within a study and across visits, and compare the common retinal area. The software also allowed for rejection of images in which the alignment was offset by more than 25 pixels or if more than 33% of the image had no valid data points, that is, the polynomial model used to fit the *z*-profile gave results outside of acceptable limits. This typically happened for areas of hemorrhage, exudate, or detachment. Images that were blurry or with uneven illumination were excluded from the analysis. Group MEIs were calculated for each set of baseline images and compared to MEIs from images taken 72 h after treatment.

Ocular Distribution of Nepafenac

^{14}C-Nepafenac 9[aniline ring-U-^{14}C] (amfenac amide) was synthesized by Amersham Life Science (Buckinhamshire, England). It was formulated as a 0.3% ophthalmic suspension by first codissolving labeled and unlabeled nepafenac in dichloromethane and evaporating under a stream of nitrogen. The test material was then ball-milled in formulation vehicle containing carbopol and mannitol. The radiopurity of the formulated ^{14}C-nepafenac was 98.1%, as determined by reverse phase HPLC (Fig. 3). The specific activity was 114.5 μCi/mg of nepafenac.

A single topical ocular dose of 30 μL ^{14}C-nepafenac ophthalmic suspension was administered to the right eye only of NZW rabbits. At multiple selected time-points through 72 h after dosing, blood samples were obtained through the marginal ear vein ($N = 4$ rabbits per time) and processed to plasma. Immediately after blood sampling, the animals were sacrificed by intravenous administration of sodium pentobarbital. The eyes were removed after sacrifice, and aqueous humor, bulbar conjunctiva, cornea, lens, iris/ciliary body, vitreous humor, retina, and choroid were collected from the dosed eyes. In addition, aqueous humor, retina, and choroid were collected from the undosed eyes. Wet tissue weights were obtained and samples (except plasma and aqueous humor) were solublized with SOLVABLE® (Packard Instruments, Downers Grove, IL). Scintillation fluid (Ultima Gold™, Packard Instruments) was added to the solublized tissues, aqueous humor and plasma, and radioactivity was measured on a Packard Tri-Carb 2300TR scintillation counter (Packard Instruments). Tissue concentrations of total radioactivity were calculated on the basis of specific activity and tissue weights.

Materials

For topical ocular administration, nepafenac was prepared in an aqueous vehicle for preliminary studies. This aqueous vehicle consisted of carbopol 974 0.5%, BAC 0.01%, tyloxapol 0.01%, edetate disodium 0.01%, NaCl 0.4%, and mannitol 2.4%. All formulations were prepared on a weight/volume basis. VOLTAREN® Ophthalmic (diclofenac 0.1%, CibaVision Ophthalmics, Atlanta, GA,) and ACULAR® (0.5% ketorolac, Allergan, Irvine, CA) ophthalmic solution were obtained commercially. Concanavalin A was purchased from Sigma (St. Louis, MO), ketamine (KETASET®) from Fort Dodge (Fort Dodge, Iowa), and xylaxine (X-JECT SA®) from Phoenix Scientific, Inc. (St. Joseph, MO).

RESULTS

At 72 h following the injection of concanavalin A, marked increases in vitreal protein and PGE_2 synthesis were observed. Subcutaneous administration of nepafenac at a dose of 10 mg/kg/day on days −1, 0, 1, 2 and 3

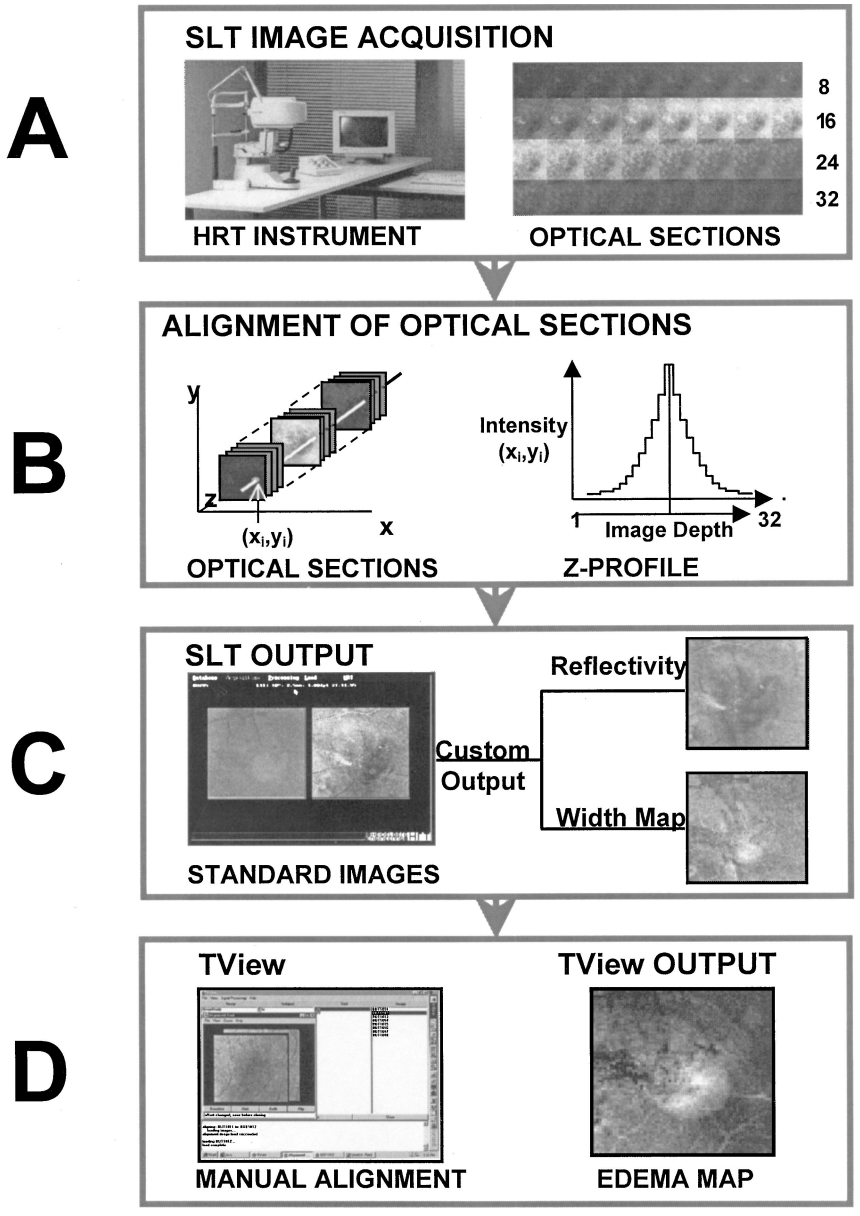


Fig. 1. Acquisition and processing images for retinal thickness using the Heidelberg Retina Tomograph and Tview Software Module. (A) The Heidelberg Retina Tomograph was used to acquire topographic images of the macula. Thirty-two optical sections were acquired along the optical axis in 1.6 s. (B) Alignment of optical sections to compensate for eye movements during image acquisition. The *z*-profile describes the resulting plot of reflectance intensity versus scan depth. (C) Standard SLT images were analyzed with the aid of custom software (Heidelberg Engineering). Image files of maximum reflectance intensity and of *z*-profile signal width (measured at 50% of the maximum reflectance intensity following fitting of the *z*-profile with a 16th order polynomial) were produced. (D) The image files were downloaded for custom analysis using Tview. Tview permitted multiple image alignment, artifact rejection, and the edema index calculation (= SW *i*/NI *i*, where SW i is the *z*-profile signal width and NI i is the normalized maximum reflectance intensity in arbitrary units, at a given pixel).

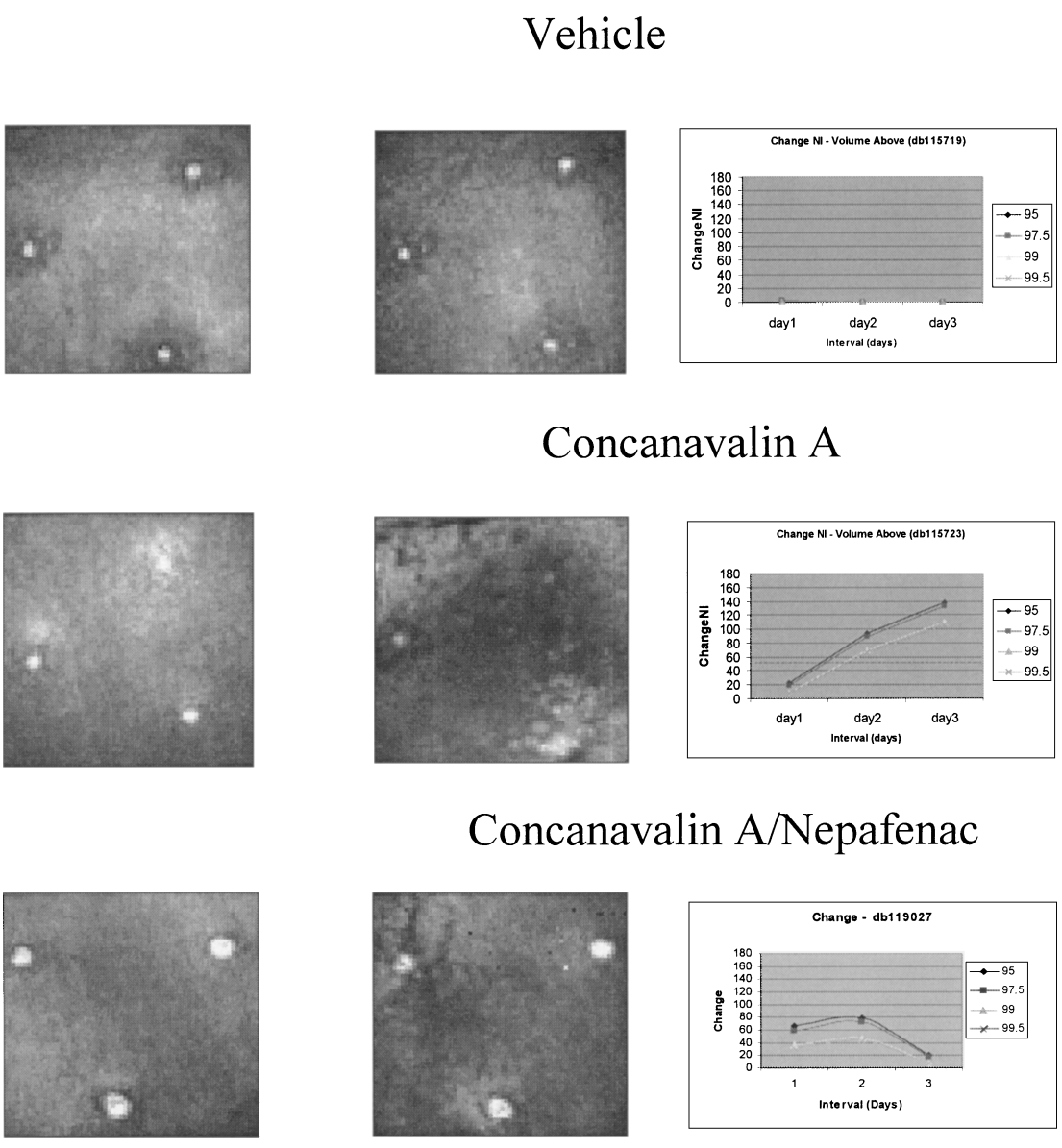


Fig. 2. Example of thickness assessment in control and nepafenac-treated animals. Illustrates typical reactions of the rabbit retina at baseline and day 3 to the experimental insult and a summary of the associated mean edema index. The top rabbit shows the reaction to an injection of saline; the middle rabbit, the reaction to an injection of concanavalin A; and the bottom rabbit, the reaction to concanavalin A and recovery following treatment with nepafenac. Note the three laser burns used as a guide for image acquisition and registration.

($n = 5$–6, Fig. 4) produced a significant (Student's t test; $p < 0.05$) reduction in retinal thickness by 88% relative to baseline levels. These reductions coincided with a measured (92%) decrease in vitreal protein levels. In a subsequent pilot study, topical application of 0.5% nepafenac in MAXIDEX® vehicle given five times per day beginning 1 day prior to intravitreal injection of 30 μg of concanavalin A resulted in a significant (65%) reduction in retinal edema (Fig. 5). This suppression coincided with an inhibition of breakdown of the blood-retinal barrier by 62%, and a 78% inhibition of the blood-aqueous barrier compared to control (Student's t test; $p < 0.05$).

In a subsequent dose–response study, nepafenac was tested for its ability to attenuate concanavalin A-mediated

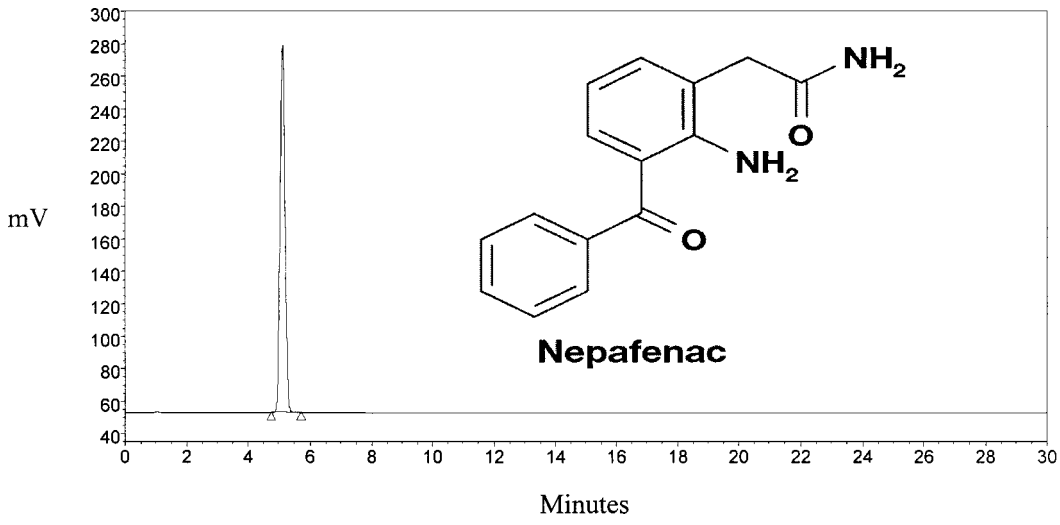


Fig. 3. Structure and representative chromatogram of nepafenac. Illustrated is the structure of nepafenac and the HPLC chromatograph of material used in pharmacodeposition studies.

changes in retinal edema index and inflammation by the topical route of administration (Fig. 6). Nepafenac (0.1, 0.5, and 1.0% in the clinically employed vehicle) was topically administered five times per day on days −1, 0, 1, 2, and 3, relative to concanavalin A injection. Composite results indicate that nepafenac significantly and dose-dependently inhibited retinal edema as assessed by retinal images obtained with the SLO. Nepafenac, at the optimally effective dose of 0.5%, prevented breakdown of the blood-retinal barrier and inhibited the production of PGE_2.

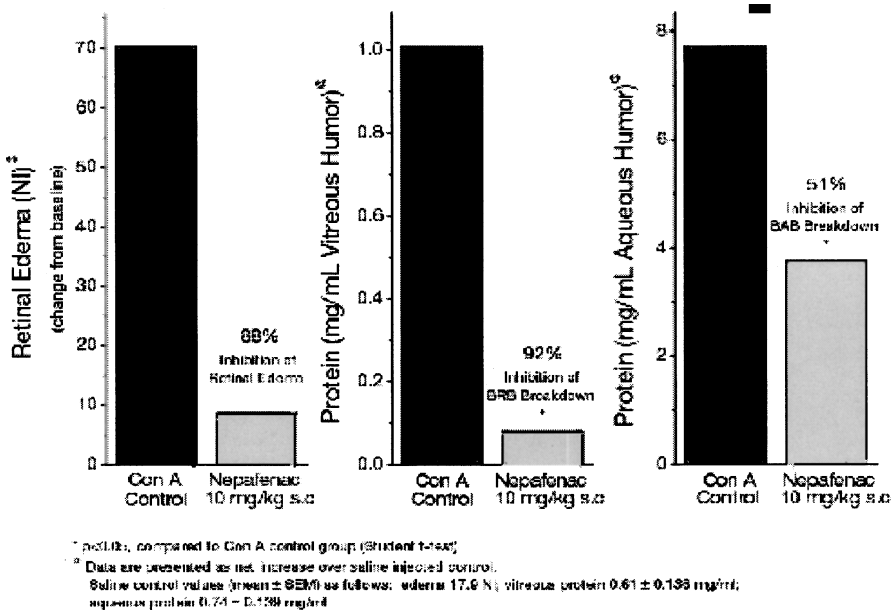
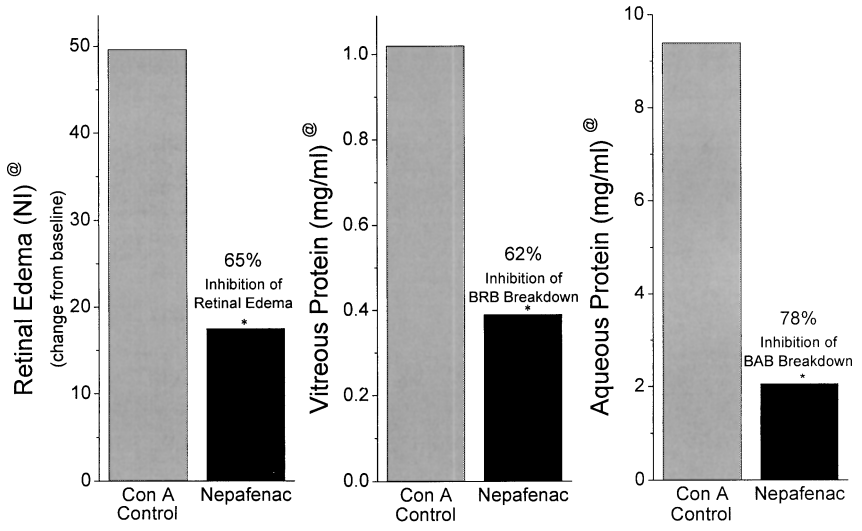


Fig. 4. Effect of nepafenac administration by subcutaneous route on retinal thickness in rabbit. In a preliminary study, the efficacy of nepafenac as an inhibitor of retinal edema (88% inhibition) was determined following subcutaneous administration of 10 mg/kg/day on days −1, 0, 1, 2, and 3 relative to intravitreal injection of concanavalin A. Data points represent means ± SEM ($n = 5$–6/group).



* p<0.05, compared to Con A control group

@ Data are presented as net increase over saline control.
Control values (mean ± SEM) as follows: edema 39.6 ± 16.45 NI; vitreous protein 0.72 ± 0.069 mg/ml; aqueous protein 0.46 ± 0.061 mg/ml

Fig. 5. Effect of topical ocular nepafenac (0.5%) on pan-retinal edema in rabbits. Topical application of 0.5% nepafenac in MAXIDEX® vehicle five times per day beginning 1 day prior to intravitreal injection of concanavalin A produced 65% reduction in retinal edema. This suppression coincided with inhibitions of 62 and 78% of blood-retinal and blood-aqueous barrier breakdown, respectively.

The efficacy of nepafenac was compared with that of Voltaren (diclofenac 0.1%) and Acular (ketorolac 0.5%) in the concanavalin A-mediated retinal inflammation model. Following topical ocular dosing, 0.5% nepafenac significantly (Student's t test, $p < 0.05$) inhibited blood-retinal barrier breakdown (46%) concomitant with almost totally suppressed PGE_2 synthesis in the posterior portion of the eye (96% inhibition). Neither Voltaren nor Acular inhibited vitreal accumulation of these markers in parallel tests (Fig. 7).

The ocular distribution of ^{14}C-nepafenac was studied in the NZW rabbit following a single topical dose of 0.3% ophthalmic suspension to the right eye only. Drug-derived radioactivity was absorbed into ocular tissues of the dosed eye, with the highest measured concentrations (C_{max}) found in the first samplings at 30 min. Relative concentrations in the anterior tissues were conjunctiva > cornea > iris/ciliary body > aqueous humor. As expected, the levels of radioactivity were lower in the posterior tissues of the dosed eye, with C_{max} in the choroid > retina > vitreous humor. The retina and choroid of the undosed eye exhibited mean maximal levels of radioactivity significantly lower than the corresponding tissues of the dosed eye (Fig. 8). Plasma total radioactivity concentrations were low (0.084 ± 0.033 μM). Pharmacokinetic parameters (C_{max}, T_{max}, and half-lives) for ocular tissues and plasma are summarized in Table 1.

DISCUSSION

Prostaglandins are involved in human intraocular inflammation (9) and released in response to ocular trauma, including surgery (10, 11). When present following trauma, intraocular surgery, or in association with uveitis, they may contribute to disruption of blood-ocular barriers and the generation of macular edema. Such is the situation for cystoid macular edema (CME), whereby visual dysfunction associated with this pathology has been linked to inflammation and the production of inflammatory mediators, including the prostaglandins (12, 13). Therapy for ocular inflammation by inhibition of prostaglandin synthesis has been practiced through the use of corticosteroids or NSAIDs; however, in the case of the former, topical corticosteroid use has been associated with elevated IOP, posterior subcapsular cataracts, and interference with postoperative wound healing (14–16). Currently, numerous clinical investigations are evaluating the intravitreal administration of steroids for the treatment of uveitis, neovascularization, and edema associated with disorders such as uveitis, postsurgical complications, diabetic

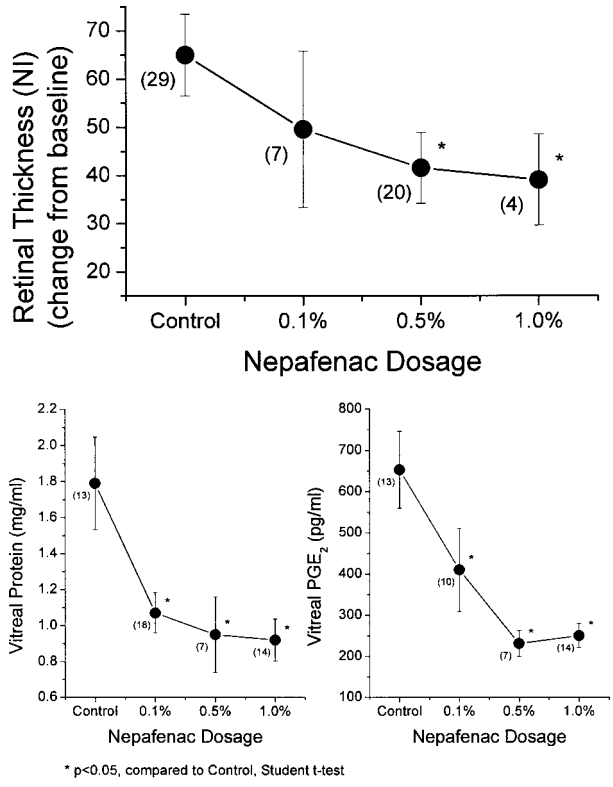


Fig. 6. Dose-dependent effect of nepafenac on pan-retinal edema in rabbits. A dose–response study assessing retinal edema and biochemical markers of inflammation was conducted as a series of separate experiments. Nepafenac (0.1, 0.5, and 1.0%) was topically administered five times per day on days −1, 0, 1, 2, and 3, relative to concanavalin A injection. Collectively, results indicate that nepafenac significantly and dose-dependently inhibited retinal edema as assessed by retinal images obtained with the SLO. It also prevented breakdown of the blood-retinal barrier and inhibited the production of PGE_2. The optimally effective dose of nepafenac was 0.5% in this study.

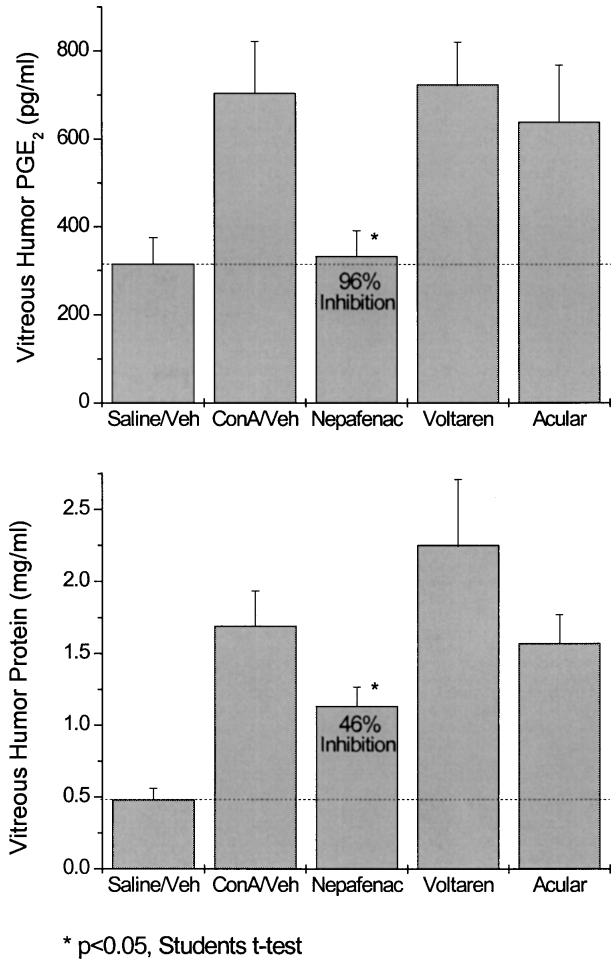


Fig. 7. Comparative efficacy of nepafenac, diclofenac, and ketorolac on pan-retinal inflammation in rabbits. The efficacy of nepafenac (0.5% in a preserved clinical vehicle) was superior to that of VOLTAREN® (diclofenac 0.1%) and ACULAR® (ketorolac 0.5%) in the concanavalin A-mediated retinal inflammation model. Following topical ocular dosing, 0.5% nepafenac significantly inhibited blood-retinal barrier breakdown (46%) concomitant with near total suppression of PGE_2 synthesis in the posterior portion of the eye (96%), while neither Voltaren nor Acular inhibited vitreal accumulation of these markers in parallel tests.

retinopathy, and AMD. As an alternative strategy, NSAID therapy has been suggested for the management of edema associated with disorders such as uveitis and diabetic retinopathy. In the laboratory, the cyclooxygenase inhibitors flurbiprofen (0.03%) and ketorolac tromethamine (0.5%) have been demonstrated to distribute to various ocular tissues following topical ocular administration in the rabbit (17, 18). In the case of flurbiprofen, tissue concentrations were found to be sufficient to inhibit prostaglandin synthesis.

Nepafenac is a nonsteroidal anti-inflammatory prodrug that potently inhibits COX-1 and COX-2 activity *ex vivo* following topical ocular administration (4). Nepafenac demonstrates low intrinsic cyclooxygenase inhibitory activity *in vitro*, yet exhibits *in vivo* efficacy equal to that of diclofenac in models of anterior segment ocular inflammation. In addition to its anterior segment efficacy, nepafenac exceeds diclofenac in its ability to reduce posterior segment ocular inflammation. *In vitro*, the compound, with an IC_{50} of 64 μM, is 530-fold less potent than diclofenac ($IC_{50} = 120$ nM) as an inhibitor of prostaglandin H synthase activity from sheep vesicular glands. *In vivo*, however, nepafenac (0.1%) exhibits anti-inflammatory efficacy comparable to that of diclofenac in a rabbit

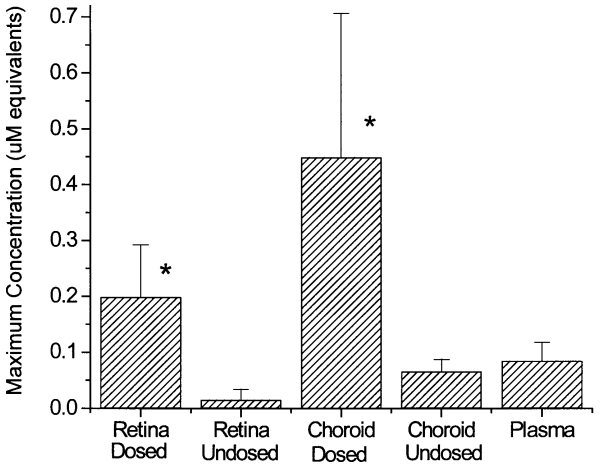


Fig. 8. Maximal concentrations of radioactivity in retina and choroid of the dosed and the undosed eyes, and in plasma (Mean ± SD, $N = 4$). $^{*}p < 0.05$ compared to corresponding tissue in the undosed eyes; exact Wilcoxon test.

model of trauma-induced breakdown of the blood-aqueous barrier (4).

In vitro metabolic and pharmacokinetic studies showed that nepafenac rapidly partitions across the cornea and sclera with a permeation coefficient sixfold greater than diclofenac and a far greater accumulation of intraocular nepafenac (5). Ke and coworkers proposed that the intraocular accumulation of nepafenac serves as a reservoir and precursor for amfenac formation by the iris/ciliary body and retina/choroid, thus permitting a prolonged suppression of ocular prostaglandin synthesis. The hydrolytic conversion of nepafenac to amfenac has been described in detail for ocular tissues of both the human and rabbit (5). The data reported showed virtually identical hydrolase distribution and specific activity for both the human and rabbit. The only difference noted was a lower hydrolase activity in human retinal tissue which appeared to be due to a loss of enzyme activity that occurred between time of death and *in vitro* assessment of hydrolase activity.

Ex vivo studies have shown that nepafenac is capable of inhibiting prostaglandin synthesis in both the anterior and posterior portions of the eye following a single topical ocular application (4). In iris/ciliary body, nepafenac uniformly inhibits the production of all prostaglandins by 80–100%, with strong suppression of PGE_2 synthesis sustained through 8 h postdosing. In contrast, diclofenac predominantly reduces PGE_2 synthesis, with diminishing activity observed 80 min through 6 h. Additionally, nepafenac reduces PGE_2 production in the retina/choroid by 66%, whereas diclofenac and ketorolac (0.5%) produce only marginal inhibition following topical drug administration.

Table 1. Pharmacokinetic Parameters for Total Radioactivity (Ocular and Systemic) Determined Following a Single Topical Ocular Dose of ^{14}C-Nepafenac 0.3% Ophthalmic Suspension to NZW Rabbits (Mean ± SD, $N = 4$)

Eye	Tissue	C_{max} (μM equivalent)	T_{max} (h)	*T1/2* (h)
	Anterior tissues			
OD (Dosed)	Bulbar conjunctiva	18.0 ± 10.9	0.5	14.3
	Cornea	15.2 ± 3.4	0.5	14.0
	Iris/ciliary body	2.50 ± 0.73	0.5	20.0
	Aqueous humor	2.25 ± 0.89	0.5	2.1
	Posterior tissues			
OD (Dosed)	Choroid	0.449 ± 0.258	0.5	2.6
	Retina	0.198 ± 0.094	0.5	2.0
	Vitreous humor	0.007 ± 0.007	0.5	2.7
	Lens	0.168 ± 0.053	0.5	22.9
OS (Undosed)	Choroid	0.066 ± 0.022	0.5	1.9
	Retina	0.016 ± 0.018	0.5	nd
	Vitreous humor	blq	nd	nd
	Lens	blq	nd	nd
	Systemic			
Plasma		0.084 ± 0.033	0.5	1.6

Note. blq: Below limit of quantification. Twice background radioactivity and equivalent to approximately 0.002–0.004 μM, depending on tissue weight. nd: Could not be determined because of insufficient data.

In this paper, systemic and topical ocular dose–response studies have been conducted in a rabbit model of concanavalin A-induced pan-retinal inflammation (6). Lectins, such as concanavalin A, have been associated with various biological actions including cytotoxicity, mitogenicity, agglutination of various cell types, stimulating the migration of tumor cells, and binding of glycoproteins and polysaccharides (19). Shier *et al.* showed that administration of small amounts of concanavalin A induced an intense and reproducible inflammatory response in experimental animals (20). In the eye, injection of concanavalin A into the vitreous elicited vascular and retinal degeneration (6, 20, 21). Intravitreal injection of concanavalin A produced marked breakdown of the blood-retinal barrier. Retinal barrier dysfunction was marginal 48 h after lectin injection but increased markedly within the next 24 h. These increases were preceded by an increase in TNFα 8 h after injection (data not shown) and were consistent with significant increases in PGE_2 in the vitreous. Gwon *et al.* (22) described inflammatory cell influx into the vitreous 72 h after intravitreal concanavalin A injection as "trace" suggesting that the retinal edema assessed 72 h after concanavalin A injection in this study is not dependent upon inflammatory cell influx. Inflammation of the posterior segment of the concanavalin A-injected eye was assessed by measuring increases in protein leakage into the vitreous, generation of edema as determined by increases in retinal thickness and increases in PGE_2 production (6). In the current study, concanavalin A-induced posterior segment inflammation and thickening (edema) of the retina were evaluated using scanning laser ophthalmoscopy (Heidelberg Retina Tomograph with software package version 2.01 b-MS; thickness determinations performed using custom software Tview) (8), and by assessing increases in vitreal protein concentrations either in the presence or absence of nepafenac. Our results showed that subcutaneous injection of nepafenac (10 mg/kg/day) given 1 day prior to, at the time of, and q.d. for 3 days following intravitreal injection of concanavalin A reduced protein levels in vitreous by 92%, and inhibited concanavalin A-induced retinal edema by 88%. In subsequent studies, nepafenac was administered topically (0.5%) in MAXIDEX® vehicle five times per day over the same period used in the subcutaneous study. In this study, nepafenac reduced the concanavalin A-induced change in baseline retinal edema by 65%. These findings suggest that nepafenac given by topical ocular administration may have a unique ability to treat posterior segment inflammation in conditions typical of diabetic retinopathy, vein occlusions, or postsurgical complications.

The ability of nepafenac to reduce posterior segment edema is unique. As a prodrug, topical administration provides a therapeutic dose that is bioavailable to the posterior segment. In the studies described within, nepafenac, given topically, was found to significantly inhibit retinal edema, prevent breakdown of the blood-retinal barrier, and attenuate production of PGE_2 in a dose–dependent manner. These findings were not duplicated using currently marketed NSAIDs by the topical ocular route of delivery. Thus, VOLTAREN® (diclofenac sodium 0.1% sterile ophthalmic solution), or ACULAR® (ketorolac 0.5%), which is topically indicated for the treatment of anterior segment inflammation or pain and photophobia, was ineffective in reducing inflammation in the posterior segment. The ability of nepafenac to affect posterior segment edema where Voltaren was inactive may, in part, be explained by the enhanced ability of nepafenac to partition across the cornea. Ke and coworkers used an *in vitro* perfusion system whose diffusible barrier was composed of corneal, scleral, and bulbar conjunctival tissues from the NZW rabbit to characterize the penetration of nepafenac (5). Under conditions of continuous drug exposure (7–117 μM), nepafenac penetrated the cornea at a rate nearly six times that observed for diclofenac.

From the ocular distribution study, it was evident that the absorption of radioactivity from topically administered ^{14}C-nepafenac was relatively rapid. The maximal measured levels in all ocular tissues and plasma occurred at the 0.5 h time-point. Total radioactivity was well distributed into both anterior and posterior tissues. Mean $C_{max's}$ in the retina and choroid of the dosed eyes were 0.20 ± 0.09 and 0.45 ± 0.26 μM, respectively. Radioactivity concentrations in these tissues declined thereafter, with half-lives between 2 and 3 h. By 4 h, the corresponding tissue concentrations were still measurable at 0.02 μM and 0.07 μm.

The maximal concentrations of radioactivity in retinal and choroidal tissues of the dosed eyes were 12- and 7-fold higher than those in the undosed eyes. Further, the levels measured in the undosed retina (0.016 ± 0.018 μM) and choroid (0.066 ± 0.022 μM) were of similar magnitude to the plasma total radioactivity concentration (0.084 ± 0.033 μM). These results are interpreted to indicate that most of the radioactivity in the retina and choroid was derived from local distribution and that a lesser component is distributed from plasma (8–14%). This may indicate that significant retinal levels can be attained through local distribution in larger species such as man. Although these results indicate topical ocular administration of nepafenac significantly inhibits retinal edema

in this animal model, results must be confirmed in human clinical trials.

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