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NEPAFENAC, A UNIQUE NONSTEROIDAL PRODRUG WITH POTENTIAL UTILITY IN THE TREATMENT OF TRAUMA-INDUCED OCULAR INFLAMMATION: II. In Vitro Bioactivation and Permeation of External Ocular Barriers

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Abstract—Nepafenac, the amide analog of the NSAID amfenac, was examined in vitro for its bioactivation by ocular tissue components and its ability to permeate external ocular barriers. Rabbit tissues catalyzed a concentration-dependent conversion of nepafenac to amfenac. The order of specific hydrolytic activity is retina/choroid ≫ iris/ciliary body. Corneal tissue showed only minimal activity. Similarly, in human ocular cadaver tissue the specific activity of iris/ciliary body was greater than cornea. Continued perfusion of the corneal epithelium demonstrated a nearly six-fold greater permeation coefficient for nepafenac ($k_p = 727 \times 10^{-6}$ min^{-1}) than for diclofenac ($k_p = 127 \times 10^{-6}$ min^{-1}). Superior permeation of conjunctival and scleral tissue by nepafenac ($k_p = 128 \times 10^{-6}$ min^{-1}) compared to diclofenac ($k_p = 80 \times 10^{-6}$ min^{-1}) was also evident. Short term perfusion (5 min) of the corneal surface with 0.1% nepafenac resulted in sustained flux of drug across the cornea for 6 h. Under identical conditions only 3.3 μM of diclofenac accumulated on the corneal endothelial side compared to 16.7 μM nepafenac. The enhanced permeability of nepafenac, combined with rapid bioactivation to amfenac by the iris/ciliary body and retina/choroid, make it a target specific NSAID for inhibiting prostaglandin formation in the anterior and posterior segments of the eye.

INTRODUCTION

Amfenac (2-amino-3-benzoylbenzeneacetic acid) is a non-steroidal anti-inflammatory drug (NSAID) with an arylacetic acid structure exhibiting potent antipyretic and analgesic properties (1). The anti-inflammatory and analgesic properties of this molecule are mediated through inhibition of cyclooxygenase (prostaglandin H synthase) (2, 3). The potent analgesic properties established in

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preclinical models have been confirmed clinically (4). A series of structural analogues of amfenac were prepared to enhance the compound's therapeutic index (3). The amide analog of 2-amino-3-benzoylbenzeneacetic acid served as a prodrug exhibiting potent anti-inflammatory activity but greatly reduced intestinal toxicity. Recently, topically administered nepafenac was shown to potently and effectively inhibit the acute inflammatory response elicited in a rabbit model of paracentesis (5). The anti-inflammatory effect was attributable to an inhibition of blood-aqueous-barrier leakage due to an effective suppression of prostaglandin synthesis of the iris/ciliary body. The long-lasting inhibition achieved was of significantly longer duration (≥6 h) than that observed following a topical ocular dose of diclofenac. Importantly, in contrast to diclofenac, topical nepafenac (0.1%) caused a significant suppression of PGE_2 synthesis in the retina/choroid, with a duration similar to that noted in the iris/ciliary body. These observations were surprising because diclofenac's cyclooxygenase inhibitory potency is 2-fold greater than that of the active metabolite amfenac.

The present report examines the in vitro hydrolysis of nepafenac by ocular tissues as well as its ability to permeate external barriers of the eye in order to account for nepafenac's paradoxically greater in vivo efficacy and longer duration of action compared to the intrinsically more potent diclofenac (5).

MATERIALS AND METHODS

Animals. New Zealand Albino rabbits were obtained from Myrtle's Rabbitry, Thompson Station, Tennessee. All aspects of handling, housing and experimentation conformed to 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). All other chemicals were either from Sigmal Chemical Company 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).

In vitro Tissue Permeation. In vitro corneal drug permeation studies were conducted as follows. Freshly excised cornea, sclera and bulbar conjunctiva were obtained from 6-week old NZA rabbits (1.3–1.5 kg). Corneas were mounted in a modified leucite perfusion chamber, in which the horizontally located donor and receiver compartments were separated by the cornea, as previously described by Schoenwald (6). The avergae corneal surface area was 1.089 ± 0.156 cm^2 (mean ± *SD*, $N = 5$). The cornea was oriented such that the epithelial cell layer faced the donor compartment. Sufficient scleral tissue remained on the tissue specimens to serve as a seal following clamping of the tissue between the cylindrical donor and receiver compartments. The attached scleral tissue surface area in contact with the incubation buffer typically corresponded to about 15% of the total tissue surface area. The clamped corneal preparations were then equilibrated in glutathione-supplemented bicarbonated Ringer (GBR) buffer. GBR buffer (pH 7.6) was prepared by mixing equal portions of one buffer solution containing 223 mM NaCl, 9.64 mM KCl, 1.57 mM NaH_2PO_4, 2.08 mM $CaCl_2$, and 1.56 mM $MgCl_2$ with a second buffer supplemented with 58.42 mM $NaHCO_3$, 10.0 mM glucose and 0.3 mM glutathione. After mixing, the GBR buffer was equilibrated for 15 min with O_2/CO_2 (95/5%).

Glutathione-supplemented bicarbonated Ringer buffer (7 ml) was added to both the donor and receiver side of the perfusion chamber. The buffer in the donor (epithelial) side contained test compound at concentrations indicated in the results section. Immediately following addition of GBR buffer to each chamber, circulation was started by bubbling of O_2/CO_2 (95/5%) through both solutions (3–5 bubbles/sec). Samples of 0.1 mL were withdrawn from both chambers at the beginning of the experiment and at 20 to 30 min time intervals thereafter through 6 h. Samples were mixed with an equal volume of methanol and immediately subjected to HPLC analysis. Corneal functionality was routinely checked at the end of the experiment by gravimetrically determining the degree of corneal hydration. Data obtained with corneal tissue exhibiting a water content of >80% at the end of the experiment were discarded.

When drug permeation rates were assessed for scleral and conjunctival tissue from the NZA rabbit the following protocol was followed. Freshly dissected inferior, bulbar tissue was mounted in a modified Franz vertical diffusion chamber (7) containing sufficient tissue to serve as a seal upon clamping the tissue between the upper (donor) and lower (receiver) compartment. Glutathione-supplemented bicarbonated Ringer buffer solution (4.5 ml) was added to the receiver compartment, whereas GBR buffer containing test compound was added to the donor compartment (0.5 ml). The tissue permeation surface area of the perfusion chamber was 0.196 cm^2.

At selected time points corneas were removed from the perfusion chamber and immediately placed in 0.5 ml methanol. The tissue was homogenized, and proteinaceous material removed by centifugation. An aliquot (50 μl) of the clear supernatant was subjected to HPLC analysis in order to determine drug concentrations.

Sample analysis was achieved on a 25 cm × 4.6 mm I.D. Spherosorb RP-18, 10 μ column (Phenomenex Company) at a flow rate of 2.0 ml/min. Nepafenac and amfenac was separated by isocratic elution with a solvent mixture composed of acetonitrile/water/phosphoric acid (380/614/6.2 (v/v/v) (pH = 3.0)). Diclofenac elution was carried out isocratically with a solvent mixture of acetonitrile/water/phosphoric acid (500/490/10 (v/v/v) (pH = 3.0)). When conducting nepafenac and amfenac sample analysis, the column effluent was monitored spectrophotometrically via a flow detector at both 240 and 380 nm. Diclofenac sample analysis was carried out at a wavelength of 220 nm.

Under the chromatographic conditions employed and as reported elsewhere (8), amfenac spontaneously lactamized under acidic conditions to 7-benzoyl-2-oxindole. Nepafenac, on the other hand, remained structurally unchanged during chromatography. HPLC-eluted amfenac and 7-benzoyl-2-oxindole were quantified by converting their respective peak areas into mass units with the aid of a response factor (area units/μg). Nepafenac, amfenac, and diclofenac response factors were determined in separate experiments from calibration standard curves. Drug permeability coefficients (k_p) of corneal, conjunctival and scleral preprations were calculated as previously described by Grass and Robinson (9).

In vitro Metabolism. NZA rabbits (1.3–1.5 kg) were euthanized with an overdose of sodium pentobarbital into the marginal ear vein. Eye globes were immediately enucleated, cornea, retina/choroid and iris/ciliary body quickly excised, weighed and placed into incubation tubes containing 0.5 ml of GBR buffer (pH 7.6) supplemented with nepafenac. The tubes were placed in an incubation shaker and incubated for 4 to 6 h at 36°C. Samples of 0.1 ml were removed for HPLC analysis at the onset of the incubation and at pre-determined intervals (60 to 70 min) thereafter. Conditions for HPLC and quantitative assessment of the conversion of nepafenac to amfenac have been described above.

When metabolic conversion (hydrolysis) of nepafenac to amfenac in human ocular tissue was examined, eyes enucleated within 10 h post-mortem were used. Eyes were maintained on ice in a moist-chamber during shipment (<10 h). Upon arrival, cornea, iris/ciliary body and retina/choroid were immediately dissected and incubated with nepafenac as described above. Metabolic conversion of nepafenac to amfenac was assessed by HPLC as described above.

RESULTS

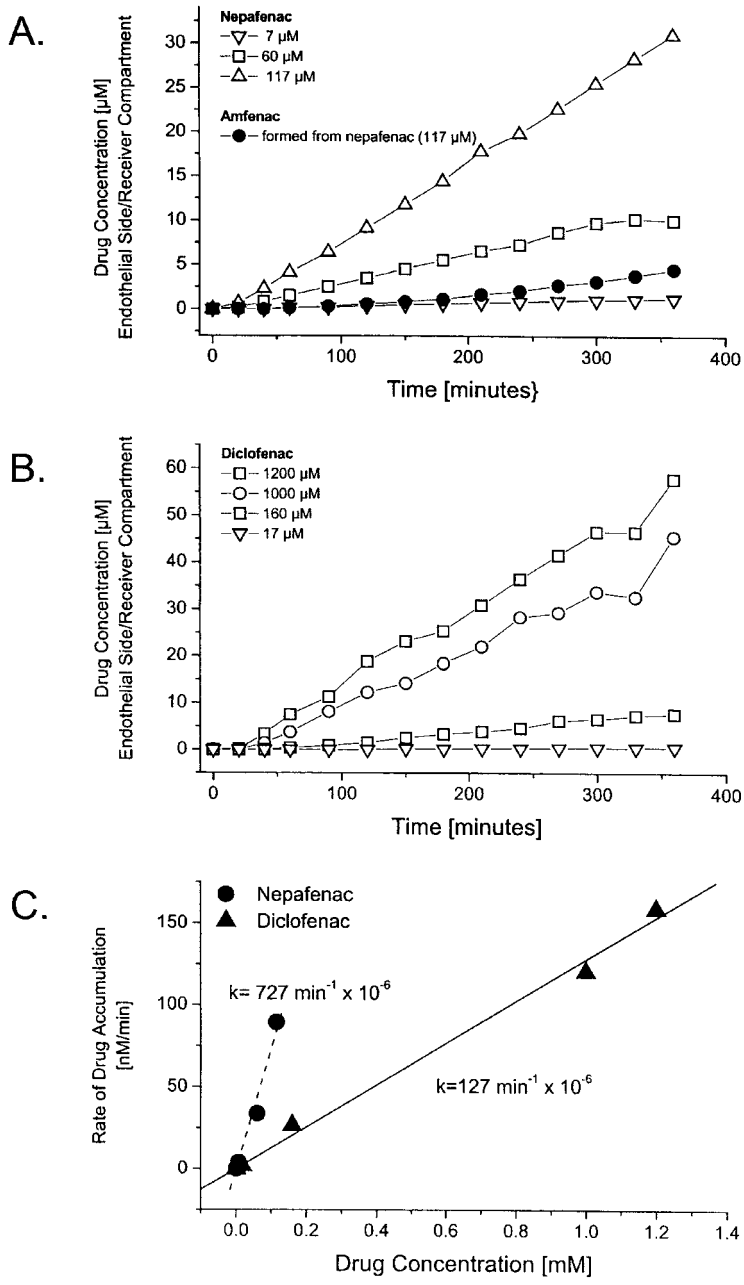
In Vitro Ocular Drug Permeation. Continuous exposure of the corneal epithelial surface (donor side) to solutions containing various concentrations of nepafenac over a period of 6 h showed that there was an initial lag prior to detecting drug on the endothelial (receiver) side. The lag period remained relatively constant (17.7–19.9 min) for the nepafenac concentrations tested (7, 60, and 117 μM). Following this initial lag period, there was a linear, time-dependent accumulation of drug on the endothelial side (receiver side) of the corneal preparation (Figure 1A). The drug flux across the cornea increased with increasing drug concentrations. At the highest concentration (117 μM), there was detectable conversion of nepafenac to amfenac by corneal tissue, with appearance of amfenac on the corneal endothelial side after a lag period of 122 min (Figure 1A—solid triangles). Thereafter, amfenac accumulation on the endothelial side progressed linearly at a rate of 18.5 nM/min. The rate of nepafenac accumulation on the endothelial side of the perfusion chamber was ≈5-fold greater (91 nM/min). Notably, incubation of nepafenac in GBR buffer alone for a period of 360 min produced no detectable quantities of amfenac (data not shown).

Corneal permeation with the reference compound diclofenac was also assessed (Figure 1B). Analogous to nepafenac, corneal permeation with diclofenac increased with increasing drug concentrations. In contrast to nepafenac, notable lag periods were observed at lower concentrations prior to detecting diclofenac on the endothelial side of the perfusion chamber. The lag periods decreased from 94 min to 58 min, 29 min and 11 min, respectively, as the initial concentration of diclofenac on the donor-side was increased from 17 μM to 160 μM, 1000 μM and 1200 μM.

From the concentration-dependent changes in rates of drug accumulation on the corneal endothelial side of the perfusion chamber (Figure 1C) first order corneal permeation rate constants (k_p) were obtained by regression analysis. Nepafenac exhibited superior corneal permeation properties with a permeation rate constant ($k_p = 727 \times 10^{-6}$ min^{-1}) ≈6-fold greater than that of the free carboxylic acid-containing NSAID, diclofenac ($k_p = 127 \times 10^{-6}$ min^{-1}).

To more closely mimic in vivo corneal drug penetration following a topical

Fig. 1. Effect of concentration on the in vitro corneal permeation of nepafenac (A) and diclofenac (B) in freshly excised tissue from the NZA rabbit. Drug, dissolved in GBR buffer (7 ml; pH 7.6), was added to the donor side (corneal epithelial side) of the modified perfusion chamber. The receiver side (corneal endothelial side) contained an equal volume of GBR buffer. Drug accumulation in the receiver side was determined over a period of 360 min by HPLC analysis of 0.1 ml aliquots removed at regular 30 min intervals. The change in rate of corneal penetration as a function of drug concentrations is depicted in (C).


A.
Nepafenac
7 μM
60 μM
117 μM
Amfenac
formed from nepafenac (117 μM)
Drug Concentration [μM]
Endothelial Side/Receiver Compartment
Time [minutes}
B.
Diclofenac
1200 μM
1000 μM
160 μM
17 μM
Drug Concentration [μM]
Endothelial Side/Receiver Compartment
Time [minutes]
C.
Nepafenac
Diclofenac
k= 727 min-1 x 10-6
k=127 min-1 x 10-6
Rate of Drug Accumulation [nM/min]
Drug Concentration [mM]

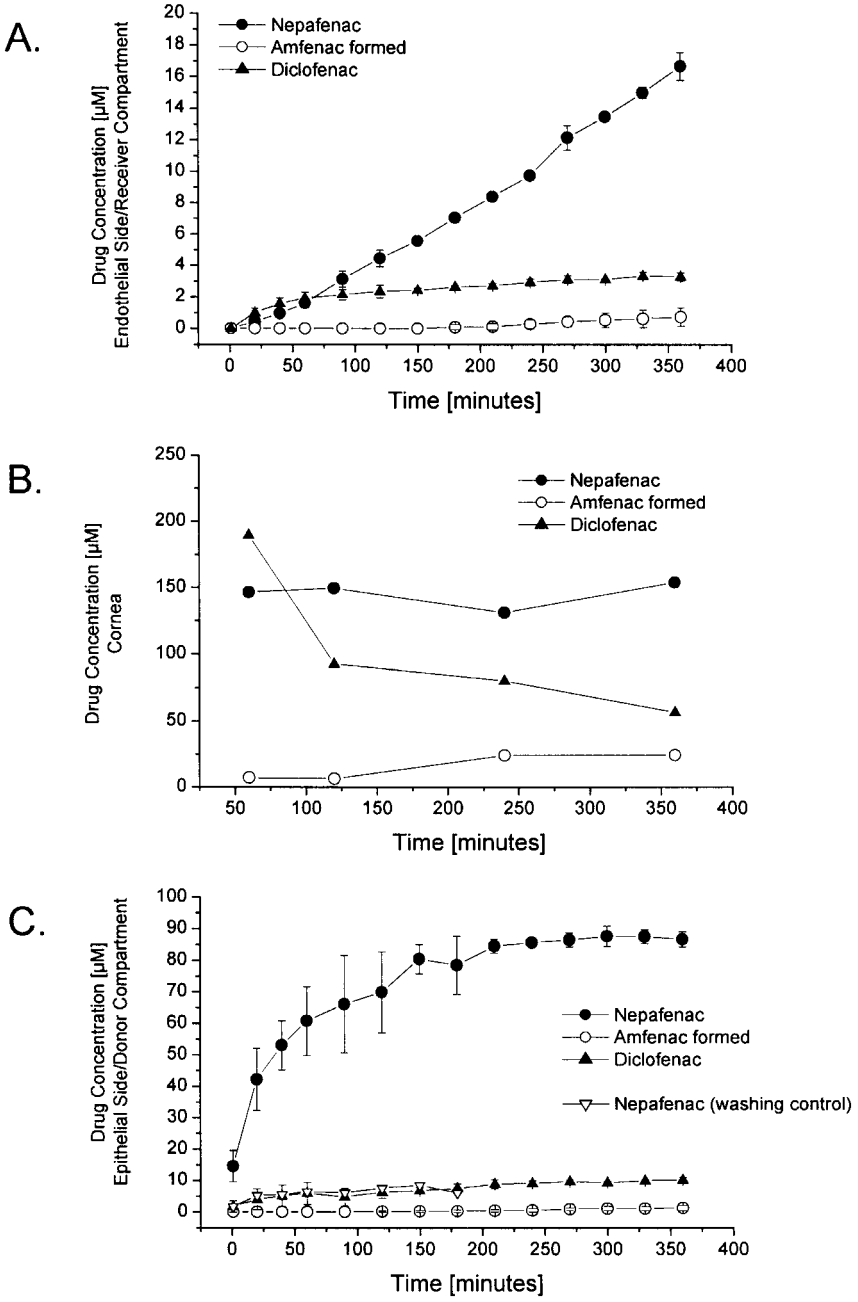
ocular dose, an in vitro study was conducted in which corneal tissue was only briefly exposed (5 min) on the epithelial side to GBR buffer supplemented with either nepafenac (0.1% suspension) or diclofenac-sodium (0.1% solution). After this short-time drug exposure the medium from the epithelial side of the perfusion chamber was removed, and the compartment was quickly washed seven times with 7.0 ml of GBR buffer. New buffer was added to the chamber, and drug appearance was monitored both in the receiver and donor compartments, as well as in corneal tissue.

Within 20 min following corneal exposure to nepafenac, drug was detectable on the endothelial side of the corneal preparation (Figure 2A). Drug then accumulated linearly on the endothelial side at a rate of 50 nM/min for the remainder of the experiment (340 min). The final concentration of nepafenac achieved on the endothelial side of the compartment was 16.7 μM. Accumulation of amfenac was evident only after 180 min and continued for the duration of the experiment at $\approx 1/13$th the rate of nepafenac accumulation (3.8 nM/min).

Diclofenac, in comparison, exhibited a somewhat slower initial rate of drug accumulation (31 nM/min) on the endothelial side of the perfusion chamber (Figure 2A). This rate of flux declined progressively with time, with drug concentrations approaching a plateau level of 3 μM within 60 min after the short term corneal drug exposure.

Concurrent assessment of drug concentrations in the cornea showed a significant accumulation of nepafenac 60 min after drug exposure (145 μM; Fig. 2B). Surprisingly, there was little change in corneal nepafenac concentration over the course of the experiment. Moreover, amfenac was detected in corneal tissue within the first 2 h of the study. However at 4 h, tissue concentrations of amfenac increased to 23.8 μM, with little change through 6 h (24 μM). In contrast, corneas exposed to a 0.1% solution of diclofenac exhibited a higher initial tissue drug concentration (190 μM at 60 min) than corneas exposed to a 0.1% suspension of nepafenac. This tissue concentration decreased rapidly to 92 μM over the next 60 min, then declined slowly to 56 μM over the remaining 4 h of the experiment.

Fig. 2. In vitro corneal drug permeation after a 5-min exposure (epithelial side) of freshly excised cornea from the NZA rabbit to a 0.1% suspension of nepafenac or a 0.1% solution of diclofenac-sodium in GBR buffer. After exposure, the donor compartment was rapidly washed with seven, 7.0 ml volumes of non-drug containing GBR buffer. Fresh (non-drug containing) GBR buffer (7.0 ml) was added to the donor compartment (epithelial side) and drug concentrations were determined in the receiver side (endothelial side) (Panel A), corneal tissue (Panel B), and donor side (epithelial side) (Panel C), at the time points indicated. Data plotted in panels A and C represent the mean ± *SD* for the time period of a) 1–60 min ($N = 5$), b) 90–120 min ($N = 4$), c) 150–240 min ($N = 3$); and the mean ± range for the time period of 270–360 min ($N = 2$). Data shown in Panel B for the time period 60–240 min represent results of a single determination, whereas the 360 min time point represents the mean ± range of duplicate determinations.


A.
Nepafenac
Amfenac formed
Diclofenac
Drug Concentration [µM]
Endothelial Side/Receiver Compartment
Time [minutes]
B.
Nepafenac
Amfenac formed
Diclofenac
Drug Concentration [µM]
Cornea
Time [minutes]
C.
Nepafenac
Amfenac formed
Diclofenac
Nepafenac (washing control)
Drug Concentration [µM]
Epithelial Side/Donor Compartment
Time [minutes]

Following the initial short-term exposure of the cornea to nepafenac and subsequent extensive washing of the donor compartment, there was a progressive re-appearance of drug in the donor compartment (Figure 2C). The concentration of drug accumulating in this compartment over the course of the study in the nepafenac exposed corneas (≈85 μM) was about 8-fold greater than that observed in corneas exposed to diclofenac (≈10 μM). Lower concentrations of amfenac (≤0.35 μM) were detectable in the donor compartment at the end of the experiment. To ensure that the re-appearance of drug was not an artifact of nonspecific nepafenac binding to the leucite material of the donor compartment, a control experiment was carried out in which the corneal tissue was replaced by a layer of parafilm. Following replacement of the parafilm, washing (7 × 7 ml GBR buffer), and addition of fresh, non drug containing GBR buffer to the donor chamber, there was only a minimal re-appearance of nepafenac in the donor compartment. The nepafenac concentration observed following washing (8.4 μM) was similar to that observed with diclofenac (10.1 μM). These results indicate that the increase in drug concentration in the donor compartment is the result of a dissociation of corneal-bound drug rather than a non-specific binding to the leucite material of the perfusion chamber.

Drug permeability of the conjunctiva and sclera was also investigated using tissue isolated from the inferior, bulbar region of the NZA rabbit. Drug flux rates were determined under conditions of continuous exposure of the epithelial tissue to drug solutions of nepafenac and diclofenac. Using either conjunctival or scleral tissue specimens there was an initial lag of about 20 min before either nepafenac or diclofenac could be detected on the receiver side (Figure 3A,B). As observed with corneal preparations, there was a subsequent linear increase in drug concentration on the receiver side of the perfusion chamber. Although nepafenac produced lower flux rates at the test concentration of 114 μM than diclofenac at 965 μM, the permeation rate constant (k_p) calculated for nepafenac was 1.6-fold greater ($k_p = 128 \times 10^{-6}$ min^{-1}) than that calculated for diclofenac ($k_p = 80 \times 10^{-6}$ min^{-1}). Notably, there was no detectable accumulation of amfenac in the receiver compartment of the perfusion chamber, indicating a lack of hydrolase activity in either conjunctival or scleral tissue.

Ocular Metabolism. With evidence for limited enzymatic conversion of nepafenac to amfenac from corneal perfusion studies (Figure 1A) and no conversion of nepafenac to amfenac in conjunctival and scleral perfusion studies, it was of interest to determine whether or not other ocular tissues of the rabbit might contribute to the hydrolysis of nepafenac following penetration of the cornea and accumulation in the aqueous humor. To address this, the in vitro hydrolysis of nepafenac was assessed using freshly isolated tissue slices of iris/ciliary body, retina/choroid and cornea from the NZA rabbit. Similar studies were executed with tissues isolated from human cadaver eyes.

Using ocular tissue from the NZA rabbit and from human cadaver eyes,

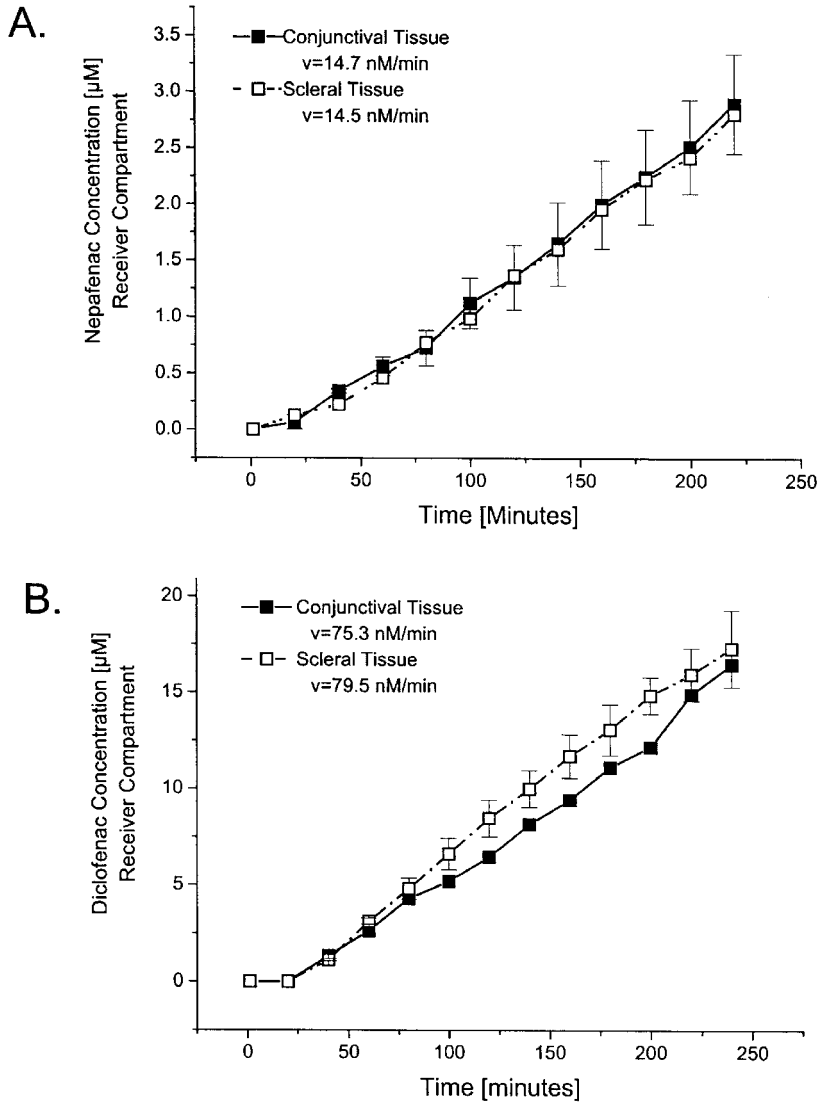


Fig. 3. In vitro permeation of (A) nepafenac (114 μM) and (B) diclofenac (965 μM) across freshly dissected inferior, bulbar conjunctival and scleral tissue from the NZA rabbit. Drug permeation was monitored at regular intervals (20 min) by HPLC analysis of aliquots (0.1 ml) removed from the receiver side following exposure of the epithelial tissue layer to drug solution (donor side). The initial volumes of both donor and receiver sides of the chamber were 0.5 and 4.5 ml, respectively.

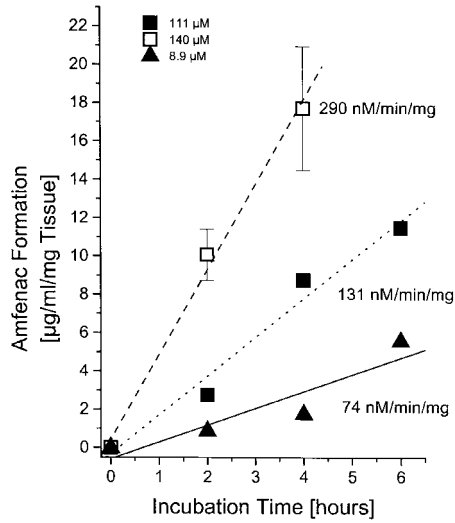


Fig. 4. Time and concentration dependent increase in the in vitro hydrolysis of nepafenac by the cornea of the NZA rabbit. Corneas were freshly dissected and incubated at 36°C in 0.5 ml of GBR buffer containing 8.9 μM, 111 μM, or 140 μM nepafenac. Aliquots (0.1 ml) were removed at the onset, or after 2, 4 and 6 h of incubation. Amfenac formation was quantified by HPLC analysis.

hydrolysis of nepafenac could be demonstrated in the cornea, iris/ciliary body and retina/choroid (Table 1). As shown in Figure 4, amfenac production progressed linearly in a concentration-dependent fashion over a period of 4 to 6 h. Rates of hydrolysis increased with increasing nepafenac concentrations. Cornea exhibited the lowest specific activity of the rabbit ocular tissues tested, followed by the iris/ciliary body and the retina/choroid. Retina/choroid was 9- and 22-fold more active than iris/ciliary body and cornea, respectively. Notably, human hydrolase activity of the iris/ciliary body and cornea was somewhat lower than that in corresponding rabbit tissues. However, hydrolase activity from the retina/choroid of human cadaver tissue represented only a fraction (2.1%) of that determined for the rabbit.

DISCUSSION

In the present investigation we examined the in vitro penetration of nepafenac across isolated external barriers of the eye as well as its bioactivation to amfenac by intraocular tissue hydrolases.

In vitro permeation studies of the sclera and conjunctiva from the NZA

Table 1. In Vitro Hydrolysis of Nepafenac by Cornea, Iris/Ciliary Body, and Retina/Choroid Tissue Slices

		Species	
		Rate of amfenac formation [pM/min/mg wet weight][a] (n)	
Ocular tissue	Test concentration [μM]	NZA Rabbit	Human
Cornea	8.9	74 (1)	26 ± 7[1,2,3] (3)
	111	131 (1)	107 ± 47[3,4] (3)
	140	290 ± 52 (2)	—
Iris/Ciliary Body	8.9	154 (1)	39 ± 4[1,2,3] (3)
	111	685 (1)	454 ± 159[4,5] (3)
	140	480 ± 94 (2)	—
Retina/Choroid	140	6400 ± 1259 (2)	135 ± 6[6] (2)[b]

[a]Rates were assessed by slope analysis at a 95% confidence limit.
[b]Mean ± range.
[c][1]Donor 1; [2]Donor 2; [3]Donor 3; [4]Donor 4; [5]Donor 5; [6]Donor 6.

rabbit indicated a 1.6-fold greater permeation rate constant for nepafenac (K_p = 128 × 10^{-6} min^{-1}) than for the reference compound diclofenac (80 × 10^{-6} min^{-1}). A greater distinction between the drugs was apparent in the cornea where the epithelial cell layer provides the rate limiting step in corneal drug absorption (9, 10). There was little resistance to corneal penetration of nepafenac, which exhibited a permeation rate constant of 727 × 10^{-6} min^{-1}. In contrast, the more polar diclofenac exhibited a much slower corneal penetration with a permeation rate constant of 127 × 10^{-6} min^{-1}.

The superior corneal permeation of nepafenac was further demonstrated in an in vitro study more closely mimicking a situation of topical ocular drug administration. Short-time exposure (5 min) of the corneal epithelium to a 0.1% nepafenac suspension (Figure 2) yielded a 5-fold greater accumulation of nepafenac on the corneal endothelial side (16.7 μM) than was observed over the same time period with a 0.1% diclofenac-sodium solution (3.3 μM). Nepafenac's continued permeation observed under our experimental conditions appeared to be attributable to the maintenance of high steady state drug concentrations in the cornea (145 μM) that were sustained by a drug gradient which re-established following extensive washing of the donor compartment facing the corneal epithelial layer. Although the initial corneal drug concentration achieved with diclofenac was greater than that attained with nepafenac, diclofenac flux across the cornea could not be sustained due to the combination of a low permeation coefficient and a re-establishment of a drug concentration gradient insufficient to drive drug permeation. Consequently, diclofenac flux across the cornea ceased within 60

min following the initial short-time exposure to diclofenac. These observations not only indicate a reversible corneal binding for both nepafenac and diclofenac but also demonstrate that the cornea exhibits a greater binding capacity (partitioning) for nepafenac than for diclofenac. Thus, the re-appearance of significant concentrations of nepafenac in the donor compartment is the driving force that permits maintenance of saturating levels of drug in the cornea, thereby sustaining nepafenac flux into the endothelial compartment.

Notably, there was only a slow production and accumulation of amfenac either in corneal tissue or in the receiver and donor compartments of the perfusion chamber. This indicates that corneal tissue contains only low levels of hydrolase activity capable of converting nepafenac to amfenac. This conclusion was confirmed in incubations of corneal slices with nepafenac where high drug levels were accessible to epithelial, stromal, and endothelial cell layers.

A more detailed examination of the in vitro bioactivation of nepafenac in freshly isolated corneal, iris/ciliary body and retina/choroid tissue slices from the NZA rabbit demonstrated an ocular tissue hydrolase distribution of retina/choroid $\gg$ iris/ciliary body > cornea. A virtually identical pattern of specific activity was noted for the cornea and iris/ciliary body from the NZA rabbit and human cadaver eyes, with the iris/ciliary body exhibiting about a two- to four-fold greater hydrolase activity than the cornea (Table 1) at concentrations of maximum compound solubility (110 μM–140 μM). Interestingly, in the rabbit most of the ocular hydrolase activity converting nepafenac to amfenac resides in the retina/choroid. The lower hydrolase activity observed in the retina/choroid of the human relative to the NZA rabbit is likely a post-morten artifact as enzymes from this tissue decay rapidly following enucleation and storage. Enzyme decay appears to be particularly prevalent in ocular neuronal tissue upon organ storage (unpublished observations).

With the cornea serving as initial drug depot and little hydrolytic conversion of nepafenac occurring during the transit in corneal tissue, drug accumulation and target specific bioactivation to amfenac by the iris/ciliary and retina/choroid can readily be envisaged following diffusion of nepafenac into the anterior and posterior chambers of the eye. This would provide for prolonged suppression of cyclooxygenase activity in vascularized tissues of the eye, indicating an advantage of nepafenac over an NSAID with a free carboxylic acid function. Evidence obtained following a single topical ocular dose of nepafenac indicates a sustained inhibition of prostaglandin synthetic capacity in the iris/ciliary body and retina/choroid body of the NZA rabbit (5). In contrast, diclofenac exhibited only a short lived suppression of prostaglandin synthesis in the iris/ciliary body and virtually no effect on prostaglandin synthesis in the retina/choroid (5) despite an intrinsic 2-fold greater cyclooxygenase inhibitory activity than amfenac. A longer duration of action was also evident for nepafenac when assessing the suppression of blood aqueous barrier breakdown in a rabbit model of paracentesis

(5). Recent evidence from clinical studies evaluating the efficacy of nepafenac as an inhibitor of inflammation following cataract surgery indicates ocular bioavailability as evidenced by a statistically significant suppression of postsurgical flare and cell influx at all doses tested (0.03%–0.3%) (11).

In summary, nepafenac's intrinsic prostaglandin H synthase activity, ocular metabolism in rabbit and human target tissues, penetration of external ocular barriers, efficacy to inhibit trauma-induced ocular inflammation, and inhibition of prostaglandin synthesis in ocular tissues following a single topical ocular dose have been investigated in preclinical models. Results demonstrate that nepafenac exhibits superior ocular bioavailability properties relative to diclofenac. Although nepafenac requires intraocular bioactivation to become an effective prostaglandin H synthase inhibitor, its superior penetration of both cornea and scleral tissues allows for a greater intraocular drug accumulation than achieved with diclofenac. The accumulated intraocular drug serves as reservoir and precursor for continued amfenac formation by the iris/ciliary body and retina/choroid, thus permitting a prolonged suppression of ocular prostaglandin synthesis. The superior ocular penetration of nepafenac and its rapid intraocular metabolism to amfenac at target tissues readily overcome the greater prostaglandin H synthase inhibitory potency of diclofenac. Thus, nepafenac may provide advantages in anti-inflammatory efficacy in all ocular compartments and a more prolonged suppression of prostaglandin synthesis than diclofenac (5). Nepafenac has been shown to be a topically effective anti-inflammatory agent for the treatment of post-surgical inflammation.

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