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Biopharmaceutical Evaluation of Ibufenac, Ibuprofen, and Their Hydroxyethoxy Analogs in the Rabbit Eye

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Two new structural analogs, 2-(4-hydroxyethoxyphenyl)acetic acid [R3] and 2-(4-hydroxyethoxyphenyl)propionic acid [R4], along with their parent compounds, ibufenac and ibuprofen, were evaluated for their biopharmaceutical properties. The analogs represented substitution of the lipophilic isobutyl side chains of ibufenac and ibuprofen with hydrophilic hydroxyethoxy side chains. Anti-inflammatory activity was evaluated by administering drugs topically to inhibit inflammation induced by using either clove oil or arachidonic acid. The rank order of activity was ibufenac ≅ ibuprofen > R3 ≅ R4. The new compounds, R3 and R4, were highly water soluble (>60-fold) and partitioned less (<1/1500-fold) into the lipid phase when compared to ibufenac and ibuprofen. R3 and R4 each had apparent corneal permeability coefficients of 6×10^{-6} cm/sec, whereas ibufenac and ibuprofen yielded values of about 22×10^{-6} cm/sec. In an ocular pharmacokinetic study in the rabbit eye, constant concentrations of each compound were maintained on the cornea in a cylinder or well fixed to the cornea, resulting in a constant input rate. This method circumvented parallel loss routes at the absorption site including nasolacrimal drainage. From area calculations the dispositions of the compounds within the eye were described by mean residence times, steady state volumes of distributions, and clearance rates. R3 and R4 were more slowly absorbed, retained within eye tissues longer, and were cleared more slowly from the eye than ibufenac and ibuprofen. The aqueous humor concentration-time profiles were also computer-fitted to equations representing classical pharmacokinetic models. For ibufenac and ibuprofen, the entire cornea was assumed to be the net barrier for entry into the anterior chamber. Whereas, for R3 and R4, the corneal epithelium and endothelium were presumed to be the diffusional barriers into and out of the stroma, the latter treated as a compartment. Aqueous humor concentrations of each drug fit the models reasonable well and agreed with conclusions made from the use of area calculations. The drop volume method was used to measure the surface tension of each compound. Both ibufenac and

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ibuprofen were considerably more surface active than R3 or R4. The greater surface tension measured for ibufenac and ibuprofen correlated to the subjective observations of ocular discomfort for these drugs.

KEY WORDS: ocular pharmacokinetics; nonsteroidal antiinflammatory agents; rabbits; alkylphenylpropionic (or acetic) acids; surface activity.

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs), particularly indomethacin and ibuprofen, have been given orally for various intraocular inflammations as an alternative to topical steroid therapy. Recently, two orally effective NSAIDs have become available for ocular use. These are flurbiprofen sodium (0.03%) and suprofen (1%). Both agents are recommended for inhibition of intraoperative miosis, uveitis, treatment of cystoid macular edema, and inflammation following cataract surgery. Oral administration of NSAIDs has been associated with gastrointestinal ulceration, and likewise, topical application of these agents is irritating to the eye (1). For alkylphenylpropionic or acetic acid derivatives, ionization of the carboxyl functionality at one end of the molecule, and the lipophilic nature of parasubstituents at the other end of the molecule create an amphiphilic structure. This surfactant-like structure contributes to ocular irritation (2).

With the existing knowledge of potential side effects of corticosteroid therapy as well as the limitations of nonsteroidal therapy, new NSAIDs with a high ratio of antiinflammatory activity to ulcerogenic behavior are desirable. Because of corneal sensitivity, the absence of irritation and tissue damage are critical requirements to be satisfied during the development of ophthalmic drugs. To this end, hydroxyethoxy analogs of ibufenac and ibuprofen, 2-(4-hydroxyethoxyphenyl)acetic acid [R3] and 2-(4-hydroxyethoxyphenyl)propionic acid [R4] were synthesized representing replacement of isobutyl side chains of the parent molecules. Ibufenac, ibuprofen, and the relatively hydrophilic analogs, R3 and R4, were evaluated for their physicochemical properties, antiinflammatory activity, and ocular pharmacokinetic behavior.

Materials

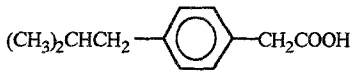
Ibuprofen, flurbiprofen, suprofen, aspirin, indomethacin, and sodium arachidonate were obtain from Sigma Chemical Co. (St. Louis, MO). Ibufenac was a gift from The Boots Co. plc (Nottingham, U.K.). [Methyl-1′,2′-^{3}H]thymidine (120 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). All the general use chemicals, including buffer

components, were used as received. New Zealand White rabbits, of either sex, were purchased from Iowa Ecology Farms (Wilton, IA).

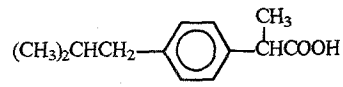
Synthesis of R3 and R4

The new compounds (R3 and R4) were prepared by *O*-alkylation of the known 4-hydroxyphenyl acetic acid and 2-(4-hydroxyphenyl) propionic acid, respectively (3). Figure 1 compares the structures of R3 and R4 to ibufenac and ibuprofen, respectively.

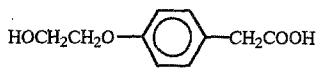
R3 was prepared as follows: A solution of methyl 4-hydroxyphenylacetate (5.00 g, 30 mmol) in 50 ml absolute ethanol was transferred to a 500-ml round bottom flask previously dried in 120°C oven overnight. To the solution was added (0.69 g, 30.0 mmol) of freshly cut metallic sodium



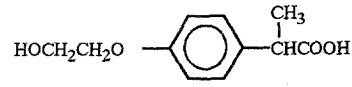
Ibufenac



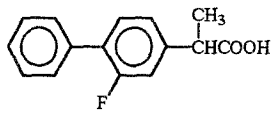
Ibuprofen



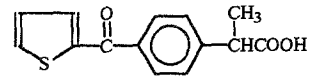
R3



R4



Flurbiprofen



Suprofen

Fig. 1. A comparison of NSAID structures. R3 and R4 are 2-(4-hydroxyethoxyphenyl)acetic acid and 2-(4-hydroxyethoxyphenyl)propionic acid, respectively.

and the mixture was heated at 80°C until the sodium dissolved. The solution was cooled to room temperature and 1.50 g (34.0 mmol) of ethylene oxide was added. The mixture was refluxed for 15 min with a dry ice condenser, cooled, and allowed to stand at room temperature for 2 hr. It was evaporated *in vacuo* after being acidified to *p*H 6 with glacial acetic acid. The residue was treated with 100 ml of aqueous 5% NaOH and stirred at room temperature for 2 hr. It was cooled in an ice bath and then acidified to *p*H 4 with concentrated HCl. The precipitated solid was collected by filtration and air dried. Recrystallization from ethyl acetate gave 3.20 g (54%) of R3, mp 139–142°C.

R4 was prepared by first adding (5.00 g, 30.3 mmol) 2-(4-hydroxyphenyl)propionic acid to a flask containing 100 ml absolute ethanol. To the resulting solution was added 1.38 g (60.0 mmol) of freshly cut metallic sodium and dissolved by heating the mixture at 80°C with continuous stirring. The solution was cooled to room temperature and 3.00 g (68.0 mmol) of ethylene oxide was added. The solution was refluxed for 15 min with a dry ice condenser. The product was isolated in an identical fashion to that cited for R3 to yield 2.10 g (32%) of R4, mp 124–126°C.

For both R3 and R4 satisfactory proton NMR spectrum, mass spectrum, and elemental analyses were obtained.

Determination of Physicochemical Properties

Determination of Percentage Purity and Melting Point

The melting points and percentage purity were determined by Differential Scanning Calorimetry (Model 2C, Thermal Analysis 3600 Data Station, Perkin-Elmer Corp., Norwalk, CT).

Determination of Solubility

The solubilities of ibufenac, ibuprofen, R3, and R4 were determined in 0.01 M hydrochloric acid. All solubility determinations were made in triplicate. Enough drug was added to 3 ml of solution to form a suspension. The suspension was sealed in a screw-capped glass vial and rotated at 20°C for 48 hr. At the end of 48 hr, a sample of saturated solution was removed and filtered using a 0.22 μm polyvinylidene difluoride filter. The first 0.5 ml was not collected. The remaining filtrate was measured for *p*H, suitably diluted and assayed for drug using HPLC methods.

Determination of Distribution Coefficient

Distribution coefficients (*DC*) were determined by utilizing the method described by Hansch (4). The aqueous phase was 0.10 M *p*H 7.65 isotonic

sodium phosphate buffer, presaturated with n-octanol for 48 hr. Similarly, n-octanol was presaturated with the aqueous phase. The drugs were dissolved in buffer at a concentration of 300 μg/ml for ibufenac and ibuprofen, 1750 μg/ml for R3, and 3500 μg/ml for R4. The drug solutions were filtered and the concentrations of the drug, prior to mixing with the organic phase, were determined by HPLC assay. The phases were mixed in conical screw-capped centrifuge tubes (Cat. no. 8062, Corning Glass Works, Corning, NY) and shaken on a vortex mixer for 15 min. The sample tubes were rotated at 20°C for 48 hr to ensure complete partitioning of drug. At the end of 48 hr the tubes were centrifuged at 1000 rpm for 30 min to separate the phases. The aqueous phase was carefully withdrawn from the tubes and a suitable dilution was assayed by HPLC. The difference in drug content in the aqueous phase before and after distribution represented the amount partitioned into the octanol phase.

Determination of pK_a

The pK_a of each drug was determined potentiometrically; the titrant was 0.0986 M sodium hydroxide. The titration apparatus consisted of a digital *p*H meter, Metrohm Herisau Multi-Dosimat (Model E-415, Metrohm AG Herisau, Switzerland), TTA80 titration assembly (Radiometer A/S Copenhagen, Denmark), combination electrode (Brinkmann Instruments, Westbury, NY), and a jacketed titration vessel (22°C). The drugs were dissolved in distilled water at a concentration of 0.45 mM for ibufenac, 0.14 mM for ibuprofen, and 1 mM for R3 and R4. The titrant was added to the drug solution in 0.015- or 0.02-ml increments so as to obtain at least 25 to 30 data points for each titration. The solution was stirred after the titrant was added and the *p*H was recorded when the measurement stabilized. The pK_a values were obtained using the Gran method for the determination of ionization constants of acids and bases (5,6).

Measurement of Surface-Active Properties

Surface-active properties were characterized by measuring surface (air/drug solution interface) tension using the drop volume method. Drop volumes of drug solutions were measured using a 2.0 ml burette attached to a micrometer (Cat. no. S-1200A, Gilmont Instruments, Div. of Barnant Co., Barrington, IL). The burette tip was modified to give a radius of 0.158 cm corresponding to a cross-sectional area of 0.0784 cm^2. The modified burette gave higher surface tension values for pure solvents such as water, methanol, and ethanol. Therefore, the burette required standardization for various concentrations of methanol in water (7) which were plotted against experimentally determined values using the same methanol concentrations in water. The corrected surface tension was then determined using the

following linear relationship:

$$\textit{Corrected surface tension} = -12.366 + 1.0552 \cdot \gamma_{\text{exptl}} \qquad (1)$$

$$R^2 = 0.996$$

where γ_{exptl} is the experimentally determined surface tension.

Using the drop volume method with the modified burette and corrected values, surface tensions of ibufenac, ibuprofen, R3, R4, flurbiprofen, and suprofen were determined. Drug solutions, identical to those used in studying eye irritation and ranging in concentration from 0.03125% to 5.00%, were placed in the 2 ml micrometer burette which measured the volume that constituted one drop of each solution. From this information and with the use of a table for corrections of drop weight surface tensions calculated by Harkins and Brown (8), the experimental surface tension was estimated using the following equation:

$$\text{Surface tension } (\gamma) = \frac{v\rho g}{2\pi r \cdot f[r/v^{1/3}]} \qquad (2)$$

where v = volume of the drop in ml, r = radius of the burette tip (0.158 cm), ρ = density of drug solution at 20°C, g = acceleration of gravity, and $f[r/v^{1/3}]$ = correction factor function. The corrected surface tension values were obtained from Eq. (1). The surface-active properties of the analog series were compared with flurbiprofen and suprofen.

In Vitro *Corneal Permeability Method*

New Zealand White rabbits, of either sex, weighing from 1.7 to 2.2 kg were sacrificed using approximately 1 ml of pentobarbital sodium 390 mg/ml and phenytoin sodium 50 mg/ml (Beuthanasia-D Special, Schering Corp., Kenilworth, NJ) by intravenous injection into the marginal ear vein. The intact eye along with lids and conjunctival sac was enucleated. Within 30 min of death, the cornea from the enucleated eye was mounted on a specially designed corneal ring (internal diameter 1.1 cm) which maintained the corneal curvature and held the eye in place. According to published procedures (9,10), the cornea was prepared and clamped within a diffusion cell which was jacketed to maintain the cornea at 37°C.

A modified bicarbonate Ringer's (BR) solution was used as the diffusion media (9). Each drug was dissolved in BR solution and placed on the donor side in concentrations ranging from 60 to 500 μg/ml. A volume of 0.15 ml from a total of 7.0 ml was removed from both the donor and receiver cells

at specified periods for assay. Equal removal from both donor and receiver cells maintained an equal hydrostatic pressure on either side of the cornea and also allowed for calculations to insure that sink conditions prevailed throughout the experiment (i.e., receiver side was always less than 5.5% of donor side). At the end of the experiment, the cornea was carefully removed, trimmed of excess scleral tissue, weighed, dried at 85°C overnight, and weighed again to determine percentage hydration.

Evaluation of Antiinflammatory Activity

A comparison of the antiinflammatory activity of the NSAIDS was conducted in two models: a clove oil chemotaxis model (11) and an arachidonic acid antiinflammatory model (12).

Clove Oil Chemotaxis Model

This model was developed by Leibowitz *et al.* (11) and depends upon *in vivo* tritium labeling of polymorphonuclear leukocytes (PMN) and the subsequent migration of PMNs to the site of inflammation, the cornea. Inflamation is induced by an intrastromal injection of clove oil. An effective NSAID given either before, during, or after induced chemotaxis will inhibit the migration of the labeled PMNs into the cornea, the latter of which can be quantitated and compared to a control.

Forty-five New Zealand White rabbits of either sex, weighing between 2.00 and 2.25 kg, were selected and randomly divided into three groups of 15 rabbits, one group for each of three treatment groups. The treatment groups represented drug treatment initiated 48 hr before (Group 1), immediately (Group 2), or 48 hr after (Group 3) injecting clove oil into the stroma, respectively. Each treatment group was further subdivided into four groups of 4, 4, 4, and 3 rabbits. Ibuprofen, R3, and prednisolone acetate were tested on the first groups of 4 rabbits, and the remaining group of 3 rabbits was given the control vehicle. In a second experiment, ibufenac, ibuprofen, R4, and the control vehicle were tested.

All animals for both experiments were given three intravenous injections of 0.05 μCi/kg of tritiated thymidine in the marginal ear vein at 24 hr intervals. Prior to inducing inflammation, each animal was anesthetized by intramuscular injection of a solution containing 120 mg/kg of ketamine hydrochloride (Ketaset®, Aveco Company Inc., Fort Dodge, IA) and 12 mg/kg of acepromazine (PromAce®, Fort Dodge Laboratories Inc., Fort Dodge, IA). An intrastromal inoculation of 30 μl of clove oil was given to both corneas using a 30-gauge $\times \frac{1}{2}$-inch long needle attached to a 1 cc tuberculin syringe. Each eye was flushed with saline as the needle was withdrawn

from the cornea. The injection of clove oil was given concomitantly with the third thymidine injection. Ibuprofen was prepared as a 1% suspension, whereas ibufenac, R3, and R4 were prepared as 1% solutions in 0.10 M *p*H 7.45 isotonic sodium phosphate buffer. Prednisolone acetate ophthalmic suspension (1%) was used as received (Pred Forte®, Allergan Pharmaceuticals Inc., Irvine, CA). The buffer served as the control. All solutions were within *p*H 7.45 ± 0.1. Both eyes were topically treated by instilling 50 μl of drug preparation or control buffer every hour for 9 hr daily. To prevent eye infections, two drops of 0.5% chloramphenicol ophthalmic solution (Ophthochlor®, Parke-Davis, Morris Plains, NJ) were given to Groups 1, 2, and 3 for 3, 3, and 5 days, respectively.

After completion of the drug therapy (5 days for Group 1 and 3 days each for Groups 2 and 3), the rabbits were sacrified as described before. The cornea was removed with an 8 mm trephine (Cat. No. E31748, Storz Instrument Co., St. Louis, MO), carefully blotted, weighed, and transferred to a scintillation vial containing 1.2 ml of tissue solubilizer (Scintanalyzed* ScintiGest®, Fisher Scientific Co., Fair Lawn, NJ) and 1 ml of distilled water. The cornea was heated at 50°C for 2 hr and then left at room temperature for 22 hr. After dissolving, 10 ml of scintillation fluid (3a70B* Complete Counting Cocktail, Research Products International Corp., Mount Prospect, IL) was added to the mixture and the vials counted for about 10 min (Beckman Liquid Scintillation Counter Model LS3801, Beckman Instruments Inc., Fullerton, CA) or until 10,000 counts were reached.

Arachidonic Acid Antiinflammatory Model

In this model aspirin, indomethacin, ibufenac, ibuprofen, R3, and R4 were evaluated and compared according to the procedure described by Abelson *et al.* (12). The drugs were assessed for their ability to alleviate the symptoms induced from topical instillation of arachidonic acid. The symptoms, lid closure, and mucous discharge, were scored on a graded scale of 0 to 3+ (12). The investigator did not know which eye received test drug or control vehicle.

Forty-two New Zealand White rabbits of either sex, weighing between 2.00 and 2.25 kg, were randomly divided into seven groups of 6 each, one group for each drug tested. Sodium arachidonate 0.5%, ibufenac 1%, R3 2%, and R4 2% were solutions, whereas aspirin 1%, indomethacin 1%, and ibuprofen 1% were suspensions. Each test drug was prepared with 0.10 M *p*H 7.45 isotonic sodium phosphate buffer, the latter representing the control vehicle. Due to potential chemical instability, sodium arachidonate and aspirin preparations were prepared and used within 1 hr.

Each rabbit received 20 μl of a drug preparation in the right eye, and 20 μl of the control vehicle in the contralateral eye. Ten minutes later, 20 μl

of 0.5% sodium arachidonate were instilled in both eyes. All eyes were then evaluated for signs of inflammation every 15 min for 1 hr. R4 was also tested after multiple dosing. In this experiment 20 μl of R4 were instilled into the right eye and 20 μl of control vehicle in the other eye every half hour for eight doses. These eyes were also challenged with sodium arachidonate and inflammatory symptoms were scored as described previously.

In Vivo Topical Infusion Method

Each drug solution was exposed to the cornea using a specially designed ocular well (13). The base of the well fits the curvature of the cornea much like a contact lens. Attached to the base was a cylinder that allowed 0.7 ml of drug solution to remain in contact with 0.503 cm^2 of corneal epithelium, but excluded contact with the sclera. This method of administration allowed for a contant concentration of drug to remain in contact with a known area of the cornea.

New Zealand White rabbits of either sex, weighing from 2.00 to 2.25 kg, were anesthetized by intramuscular injection given to the right hindquarter 20 min prior to the start of the experiment. The rabbit was placed on its right side with its body below the neck secured with a large plastic bag. The inner base of the ocular well was coated with a small amount of silicone grease and placed over the sclera of the right eye. The silicone grease stabilized the well on the eye and did not come in contact with the drug solution. Two or three hemostats were fastened to the fur around the eye to position the well directly over the cornea. Each drug, dissolved in 0.10 M *p*H 7.80 isotonic sodium phosphate buffer, was placed in the well at time 0. Ibufenac and ibuprofen were studied at 300 μg/ml, whereas R3 and R4 were studied at 900 μg/ml. The final *p*H of buffer after dissolving each drug was 7.65 ± 0.05. The drug solution in the well was replaced every 5 min to maintain the initial concentration.

Six rabbits were used for each time interval for both infusion and postinfusion phases. After a specified period (5–120 min), the rabbits were sacrificed, the well removed from the eye, and the eye rinsed with approximately 5 ml of normal saline. Excess saline was gently wiped from the eye. At that time samples of aqueous humor, cornea, and iris/ciliary body were removed for analysis.

For the postinfusion study, the well or cylinder containing drug solution was removed from the cornea at 120 min. The eye was very carefully blotted but not rinsed. Groups of 6 rabbits, each representing different time intervals from 120 to 240 or 390 min for either ibufenac and ibuprofen or R3 and R4, respectively, were maintained on anesthetic. At the prescribed time, the rabbits were sacrificed and tissue samples removed for analysis of drug.

Aqueous humor samples of 150 to 230 μl were obtained by paracentesis using a 28-gauge $\times \frac{1}{2}$-inch long needle attached to a 1 cc insulin syringe. The insulin syringe was capped and placed on dry ice immediately after removing aqueous humor. Corneal samples were removed with an 8-mm trephine, carefully blotted, weighed, and placed into a 2-ml screw-capped plastic vial (cat. no. 72.693, Sarstedt Laboratory Wares, Numbrecht, Germany) on dry ice. Iris/ciliary body was removed by gently pulling on the tissue from the ocular cavity with a forceps. Iris/ciliary body was blotted, weighed, placed in a 2-ml screw-capped plastic vial. Each tissue sample was frozen for future analysis of drug by high pressure liquid chromatography (HPLC).

It was suspected that the epithelium and endothelium were significant barriers for drug penetration of the hydrophilic analogs, R3 and R4, into and out of the stroma which functioned as a compartment. Therefore, in another set of experiments R3 and R4 were infused at 900 μg/ml for 120 min, the rabbit sacrificed, and the surface of the cornea rinsed and blotted as before. The entire epithelium was removed immediately by scraping the corneal surface with the blunt end of a scalpel blade. The endothelium was removed by carefully and gently rubbing the endothelial surface with a cotton-tipped applicator. The stroma was then blotted, weighed, and placed in a 2-ml screw-capped plastic vial and frozen on dry ice.

Evaluation of Eye Irritation

In preliminary experiments of topical infusion using an ibuprofen concentration of 2000 μg/ml, the anesthetized rabbits showed mild signs of discomfort by moving their forelegs after the solution was maintained on the cornea for 1–2 min. In a separate series of experiments groups of 6 rabbits were anesthetized and eye wells were placed on the cornea as described in the procedure for topical infusion. Stock concentrations of ibufenac and ibuprofen (5000 μg/ml) were prepared in 0.10 M *p*H 7.65 isotonic sodium phosphate buffer by first dissolving the drugs in equimolar amounts of 0.10 M sodium hydroxide. From these stock solutions, concentrations of 300, 500, 750, 1000, 1250, 1500, 1750, and 2000 μg/ml were prepared in 0.10 M *p*H 7.65 sodium phosphate buffer. The *p*H ranged from *p*H 7.80 to 7.86, whereas the osmolarity ranged from 262 to 271 for ibufenac and ibuprofen and from 238 to 274 mOsm/Kg for R3 and R4, respectively. The lowest drug concentration was added to the eye cup wells and the number of rabbits that exhibited discomfort in the first minute was noted. If no discomfort was observed in all 6 rabbits, a higher concentration was tested on a new group of 6 rabbits. The rabbits that did not experience discomfort were used again after a 48-hr rest period. The rabbits for which discomfort was observed were sacrificed before regaining consciousness. In

this manner the concentration that resulted in discomfort in 50% of the test animals was considered an IC_{50}. R3 and R4 were also tested at 5000 and 10,000 μg/ml.

Analysis of Drug by HPLC

Samples of cornea, stroma, or iris/ciliary body were placed into a 3-ml tissue grinding tube (Duall® type, size 21, Kontes Scientific Glassware/Instruments, Vineland, NJ) along with 1 ml of 0.05 M sodium carbonate and allowed to stand at room temperature for 3 hr. The corneal samples were homogenized at 60 rpm (Con-Torque Power Unit, Eberbach Corp., Ann Arbor, MI) for 3 min using a fritted glass pestle (Kontes, Vineland, NJ). Similarly, iris/ciliary body samples were homogenized at 300 rpm for 3 min. Each sample was centrifuged at 4000 rpm for 30 min (IEC Centra-7R Refrigerated Centrifuge, Needham Heights, MA). The supernatant fractions were collected and 20 μl was injected into a HPLC.

The HPLC system consisted of a solvent delivery pump (Model LC-6A, Liquid Chromatograph, Shimadzu Corp., Kyoto, Japan), a variable wavelength UV-visible spectrophotometric detector (Model SPD-6AV, Shimadzu Corp., Kyoto, Japan) and a chart recorder/integrator (Model C-R3A, Chromatopac, Shimadzu Corp., Kyoto, Japan) operating at 5 mm/min. The samples were injected onto a reverse phase μBondapak® C_{18} HPLC column (Part no. 27324, 30 cm × 3.9 mm i.d., Waters Chromatography Division, Milford, MA) attached to a matching guard column unit (Guard-PAK Precolumn Module, Part no. 88141 and Part no. 88070 μBondapak® C_{18} Guard-PAK inserts, Waters Chromatography Division, Milford, MA) using a syringe loading sample injector (Model 7125, Rheodyne Inc., Cotati, CA) fitted with a 20 or 100 μl loop (Rheodyne Inc., Cotati, CA).

Structural similarities between compounds made it possible to use common assay procedures. The HPLC assay procedure developed for ibuprofen by Lockwood and Wagner (14) was modified and used as the basis for the assay development of ibufenac, ibuprofen, R3, and R4. The mobile phases, filtered and deaerated before use, consisted of varying ratios of distilled water to methanol with 1 ml of 85% w/w phosphoric acid added to each liter of mobile phase. The mobile phases which had an approximate *p*H of 4 were pumped at a flow rate of 2.0 ml/min. Relative proportions of water to methanol and assay wavelengths used for each drug are given in Table I.

To determine extraction efficiencies, a known amount of drug dissolved in 0.10 M *p*H 7.65 isotonic sodium phosphate buffer was incubated for 3 hr at room temperature with freshly excised cornea or iris/ciliary body tissues.

Table I. Mobile Phase Composition and Assay Wavelengths Used for HPLC Analysis

Compound	Proportion of water:methanol	Assay wavelength (nm)
Ibufenac	340:660	220
Ibuprofen	300:700	220
R3	700:300	224
R4	630:370	226

The tissues were then extraced with 0.05 M sodium carbonate, homogenized and centrifuged as before. The supernatant fraction was collected and analyzed for drug content using HPLC methodology. The extraction efficiencies were constant for each compound and ranged from 64.1 to 94.6% for cornea and 51.3 to 84.7% for iris/ciliary body tissues. External standards were chromatographed for each experimental determination and linear calibration curves were used to convert peak height to concentration.

RESULTS

Determination of Physicochemical Properties

Table II lists the values obtained for the physicochemical parameters, melting point, purity, solubility, distribution coefficient, pK_a, and corneal permeability.

The α-methyl, isobutyl, and hydroxyethoxy substituents have a marked effect on the intrinsic solubilities (0.01 M HCl) of the analog series. In comparing ibufenac and ibuprofen, the addition of an α-methyl group resulted in a three-fold decrease in solubility which is expected. However, when comparing the solubility of R3 versus R4, the α-methyl group was responsible for a twofold increase in solubility. Other factors, such as

Table II. Physicochemical Properties of Nonsteroidal Antiinflammatory Agents

Compound	m.p. (°C)	Purity (%)	Solubility[a] (μg/ml)	log DC[b]	pK_a	CPC[c] (cm/sec × 10^6)
Ibufenac	85–87	99.64	98.2	0.303	4.43	21.2 ± 1.36
Ibuprofen	75–77	99.53	28.7	0.806	4.54	22.4 ± 1.19
R3	140–142	99.73	1786.0	−3.55	4.38	6.22 ± 0.29
R4	120–122	97.68	4318.0	−2.38	4.47	5.97 ± 0.26

[a] Intrinsic solubility determined in 0.01 M HCl.
[b] Log of distribution coefficient (n-octanol/0.10 M pH 7.65 sodium phosphate buffer).
[c] Corneal permeability coefficient determined across excised rabbit corneas ±1 SD.

self-association due to the presence of the hydroxyethoxy substituent or the energy necessary for dissociation of the crystal lattices contribute to the complexity of explaining the solubility differences of the analogs.

The distribution coefficients depended on the substituents. The α-methyl and isobutyl groups were responsible for an increase in lipophilicity, whereas the hydroxyethoxy group contributed to the hydrophilicity. The rank order from lipophilicity to hydrophilicity is ibuprofen > ibufenac > R4 > R3 which agrees with what one would expect for these structures.

The substituent differences between the molecules had almost no effect on their pK_as which ranged from 4.38 to 4.54. Consequently, due to their similarity, pK_a is not a determinant in explaining differences in pharmacokinetic behavior.

The corneal permeability coefficients (*CPC*) were determined over a wide range of drug concentrations (60 to 500 μg/ml). There were no statistically significant differences between the mean values over the concentration range for each drug, nor were there any trends in the results. Therefore, the results were averaged over concentration and appear in Table II for each drug. Ibufenac and ibuprofen have values of 22×10^{-6} cm/sec, whereas R3 and R4 have values of 6×10^{-6} cm/sec. Clearly, the differences in partitioning result from substitution of the isobutyl group with the hydroxyethoxy group, and not the presence or absence of the α-methyl group. The log *DC* (octanol/*p*H 7.65 buffer) for ibufenac and ibuprofen are 0.303 and 0.806, which represent drugs for which the epithelium, stroma, and endothelium are about equal in their barrier contributions (15). However, R3 and R4 have log *DC* values < -2 and therefore the epithelium, and to some extent the endothelium, are significant barriers to penetration into the anterior chamber. When comparing ibufenac and ibuprofen (or R3 vs. R4), one would expect a higher *CPC* for the latter because of its higher log *DC* value. The hydration levels for excised corneas exposed to solutions from each drug (60–500 μg/ml) measured from 81 to 86%. Whereas the normal hydration level of a cornea is 76–80%, higher values mostly reflect corneal damage. As damage occurs, the interstitial spaces widen (16), the percentage hydration increases (17), and permeability becomes less of a function of drug partitioning. We used the lowest concentrations that would permit reliable assay sensitivity; nevertheless, a small degree of damage to the epithelial surface occurred likely resulting in somewhat higher *CPC* values, particularly for ibufenac and ibuprofen, making it difficult to show differences based upon subtle changes in lipophilicity from the addition of an alpha carbon (i.e., ibufenac vs. ibuprofen and R3 vs. R4).

The results for surface tension are shown in Fig. 2. From the results it is clear that ibufenac and ibuprofen are the most surface active, whereas R3 and R4 have substantially less surface-active properties as expected from

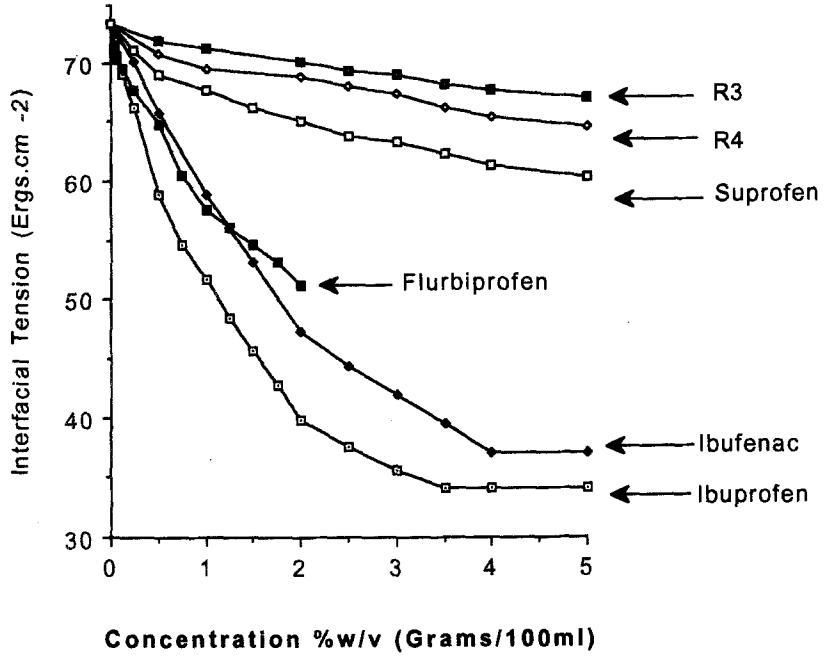


Fig. 2. Surface-activity measurements for NSAIDs. R3 and R4 are 2-(4-hydroxyethoxyphenyl)acetic acid and 2-(4-hydroxyethoxyphenyl)propionic acid, respectively.

their hydrophilic behavior. Ibuprofen has the greatest surface-active properties followed by ibufenac ≅ flurbiprofen > suprofen > R4 > R3. The surface-active properties are a contributing factor to their irritation potential (18,19) which is of considerable concern for an ophthalmic NSAID. For alkylphenylpropionic or acetic acid derivatives, parasubstituents of a less lipophilic character than an isobutyl group are most desirable providing the antiinflammatory activity of the NSAID is not significantly compromised.

Evaluation of Eye Irritation

The discomfort level of each solution affixed to the cornea with the use of the corneal well was measured by observing discomfort signs (movement of the foreleg) from an anesthetized rabbit as described in the experimental section. R3 and R4 showed no discomfort up to the highest concentration applied to the eye (10,000 μg/ml). In a study by Ellingson *et al.* (2) epithelial damage was statistically significant from topical exposure of ibufenac and ibuprofen (0.05%) over 2 hr using the topical infusion method, whereas R3 and R4 showed no differences from the control vehicle. Both

ibufenac and ibuprofen are amphiphilic due to the hydrophilic carboxylic group in one region and the tertiary butyl group in the opposite region of the molecule. Clearly, it is advantageous to develop an ocular NSAID with less surface activity while maintaining sufficient antiinflammatory potency.

Evaluation of Antiinflammatory Activity

Clove Oil Chemotaxis Model

In this model the percentage reduction in radioactivity in each cornea after treatment was taken as a measure of the drug's antiinflammatory activity. In two experiments, the cyclooxygenase inhibitors, ibufenac, ibuprofen, R3, and R4, were compared to prednisolone acetate, a steroid that inhibits both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. The results are summarized in Table III.

In Group 1, drug treatment began 48 hr before the injection of clove oil which allowed for drug to penetrate and accumulate in healthy, uninflamed cornea tissue. For all drugs, a statistically significant reduction in inflammation was observed when compared to the vehicle treatment. However, there were no significant differences among the drugs. In Group 2, clove oil and drug treatment was started simultaneously which is obviously a more stringent condition for drug to show activity. Under these conditions, prednisolone acetate, ibufenac, and ibuprofen showed statistically significant activity, but not R3 or R4. When drug treatment started 48 hr after

Table III. Antiinflammatory Activity of Prednisolone Acetate and NSAIDs Following Topical Treatment of Cornea Upon Intrastromal Injection of Clove Oil

	% reduction in inflammation				
Drug treatment	Prednisolone acetate	Ibufenac	Ibuprofen	R3	R4
Therapy started 48 hrs before inducing inflammation	$28.57^{a,c}$	$42.42^{b,c}$	$39.77^{a,c}$ $34.04^{b,c}$	$34.55^{a,c}$	$55.69^{b,c}$
Therapy started immediately after inducing inflammation	$37.31^{a,c}$	$33.48^{b,c}$	$30.49^{a,c}$ $38.85^{b,c}$	$0^{a,d}$	$3.22^{b,d}$
Therapy started 48 hrs after inducing inflammation	$25.30^{a,d}$	$26.22^{b,c}$	$14.76^{a,d}$ $28.11^{b,c}$	$0^{a,d}$	$19.98^{b,c}$

[a] The results from the first set of experiments (coefficients of variation ranged from 5.2 to 35.4%).
[b] The results from the second set of experiments (coefficients of variation ranged from 14.8 to 38.2%).
[c] Reduction in inflammation compared to control eye; statistically significant, $P < 0.05$.
[d] Reduction in inflammation compared to control eye; not statistically significant, $P > 0.05$.

the injection of clove oil (Group 3), the results showed no statistically significant activity for prednisolone acetate, ibuprofen, or R3 in the first experiment; however, ibufenac, ibuprofen, and R4 showed statistically significant activity in the second experiment. Prednisolone acetate and ibuprofen had relatively high standard deviations associated with Group 3 treatment in the first experiment and therefore were not statistically significant from the vehicle control even though their percentage reduction in inflammation was approximately the same as ibufenac, ibuprofen, or R4 in the second experiment. Ibufenac and ibuprofen have been compared in different systemic models (20) which have shown that ibufenac is slightly less effective in reducing inflammation. Overall, R3 and R4 are active when drug treatment was started either simultaneously or after the injection of clove oil. Although R3 and R4 are active, they are less active than ibufenac, ibuprofen, or prednisolone acetate.

In this model, clove oil severely disrupts the epithelial barrier which may obscure differences between hydrophilic and lipophilic drug penetration. The corneas treated with vehicle were opaque after the injection of clove oil. However, corneas treated with each drug became translucent on the second day following the clove oil injection indicating an effective response.

Table IV. Antiinflammatory Activity of NSAIDs Following Topical Treatment of Rabbit Eyes Pretreated with Arachidonic Acid

Topical treatment	Lid closure scores ($\bar{x} \pm SD$)[a]	Mucous discharge scores ($\bar{x} \pm SD$)[b]
Aspirin	0.33 ± 0.52[c]	0.17 ± 0.41[c]
Control vehicle	2.50 ± 0.84	2.33 ± 0.82
Indomethacin	0.00 ± 0.00[c]	0.20 ± 0.45[c]
Control vehicle	2.20 ± 0.45	2.20 ± 0.45
Ibufenac	1.00 ± 1.10[c]	1.00 ± 0.00[c]
Control vehicle	2.67 ± 0.52	2.33 ± 0.82
Ibuprofen	0.80 ± 0.84[c]	0.00 ± 0.00
Control vehicle	2.60 ± 0.89	1.00 ± 1.00
R3	2.17 ± 1.17	1.60 ± 0.89
Control vehicle	2.67 ± 0.52	1.80 ± 0.84
R4	1.60 ± 1.34	1.60 ± 0.55
Control vehicle	2.20 ± 0.84	2.20 ± 0.84
R4-Multiple dose	2.00 ± 0.63	2.17 ± 0.41
Control vehicle	2.33 ± 0.52	2.00 ± 0.00

[a] Peak effect (15 min) used to determine statistical significance.
[b] Peak effect (30 min) used to determine statistical significance.
[c] $P < 0.05$ compared to control vehicle, all other determinations non-significant.

Arachidonic Acid Antiinflammatory Model

In this model by Abelson *et al.* (12), the instillation of arachidonic acid in the rabbit eye produces inflammatory responses that can be easily scored. The mean results of graded scoring at the peak effect for lid closure (15 min) and mucous discharge (30 min) are presented in Table IV. The Wilcoxon sign rank test (21) was used to compare drug-treated and control eyes. Aspirin, indomethacin, ibufenac, and ibuprofen blocked lid closure significantly ($P < 0.05$). Aspirin, indomethacin, and ibufenac were also effective in suppressing mucous discharge ($P < 0.05$), but ibuprofen showed activity which was not statistically significant ($P < 0.125$). Although R3 and R4 showed minimal activity following a single dose instillation, neither analog showed a statistically significant effect; nor was R4 significantly effective following multiple instillations.

In vivo Topical Infusion

R3 and R4 were applied to the cornea at a constant nonirritating concentration of 900 μg/ml for 120 min; lower concentrations produced tissue levels below the sensitivity of the assay, particularly for iris/ciliary body tissue. Ibufenac and ibuprofen were not well tolerated when applied at concentrations above 300 μg/ml. The solution applied to the well was exchanged every five min, to maintain the applied concentration of either 300 or 900 μg/ml. Analysis of these solutions revealed that the concentration was always $\geq$96% of the original concentration. In each treated animal, the contralateral eye was also analyzed for drug in cornea, aqueous humor, and iris/ciliary body and found to be devoid of drug at the sensitivity level of the assay. Figures 3 and 4 summarize the mean concentrations of each drug in cornea, aqueous humor, and iris/ciliary body over the infusion and postinfusion time periods.

The results were consistent with the expectation that corneal concentrations and partition behavior were directly related. The concentrations of ibufenac and ibuprofen rose sharply in corneal tissue and reached an apparent steady state within 40 min. Ibufenac had lower corneal levels than ibuprofen. R3 and R4 also had lower drug concentrations than ibufenac and ibuprofen even though they were infused at three times the concentration of ibufenac and ibuprofen. R3 and R4 took a longer time ($\cong$75 min) to reach steady state indicating a larger corneal elimination half-life. R3, the most hydrophilic analog, had much lower corneal levels than R4.

Ibufenac and ibuprofen had identical aqueous humor levels even though ibuprofen had higher corneal levels than ibufenac. R3 had lower aqueous humor levels than R4 throughout infusion and postinfusion. R3 and R4 did not reach steady state in aqueous humor but because of practical

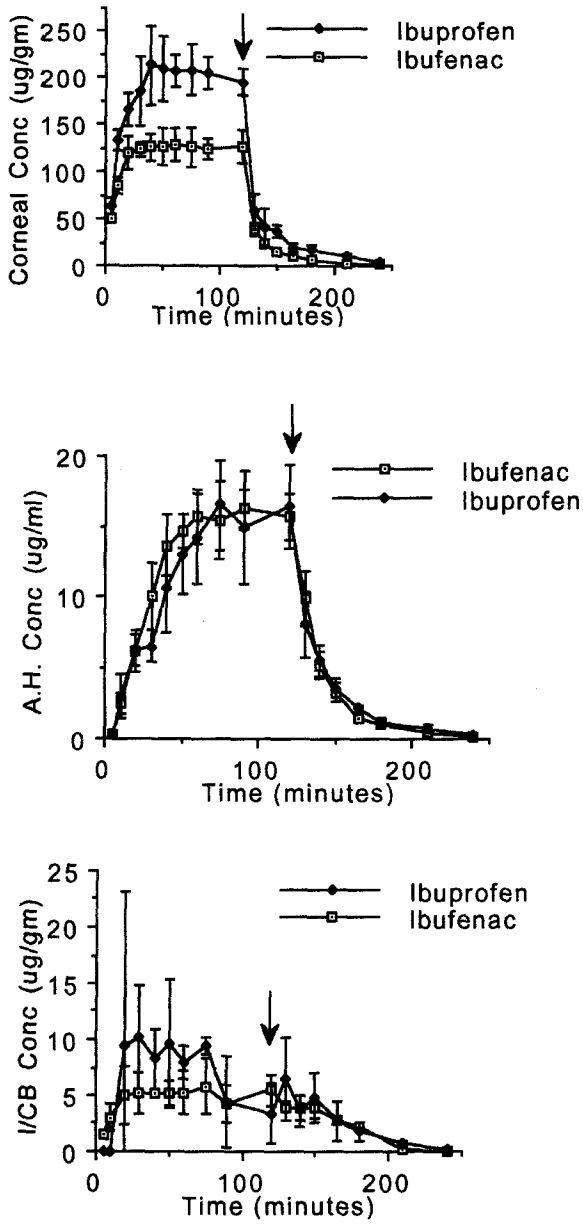


Fig. 3. Corneal, aqueous humor, and iris/cirilary body concentrations of ibufenac and ibuprofen following application of a constant concentration of drug to the cornea of anesthetized rabbits for 120 min (indicated by the arrow). Following 120 min, drug decline is measured through 240 min.

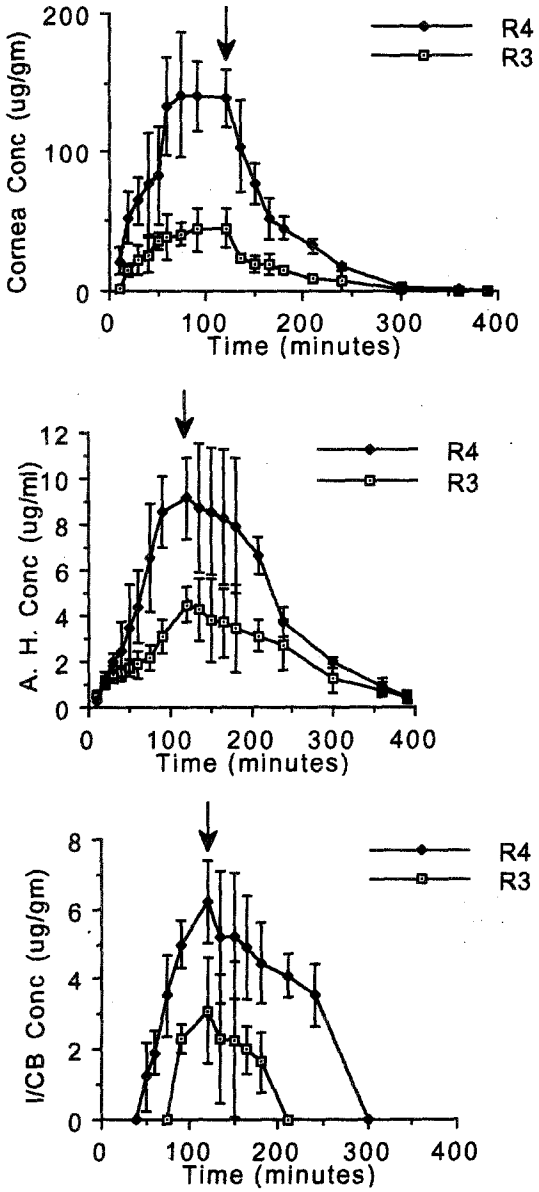


Fig. 4. Corneal, aqueous humor, and iris/ciliary body concentrations of R3 and R4 following application of a constant concentration of drug to the cornea of anesthetized rabbits for 120 min (indicated by the arrow). R3 and R4 are 2-(4-hydroxyethoxyphenyl)acetic acid and 2-(4-hydroxyethoxyphenyl) propionic acid, respectively. Following 120 min, drug decline is measured through 390 min.

problems the infusion could not be continued beyond 120 min. Once drug solutions were removed from the cornea at 120 min, drug levels in either the cornea or aqueous humor declined very sharply for ibufenac and ibuprofen. For R3 and R4, the aqueous humor decline in drug concentration was not precipitous from 120 through 210 min and suggested that the cornea was acting as a slow-release reservoir.

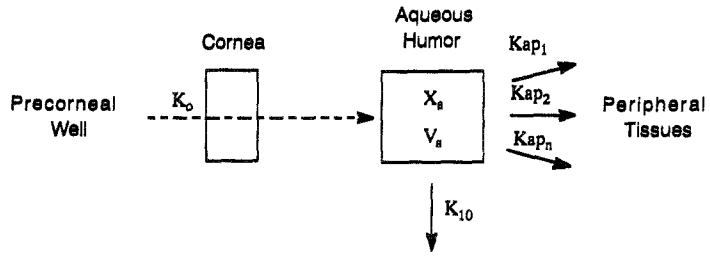
For all drugs and in particular for R3 and R4, the levels in the iris/ciliary body were low as expected. At 120 min of infusion, the aqueous humor to iris/ciliary body concentration ratios for ibufenac, ibuprofen, R3, and R4 were 2.87, 4.80, 1.45 and 1.47, respectively. These results correlate very well to the log *DC* values for the drugs.

Since ibufenac and ibuprofen had log *DC* values > 0 and also exhibited moderately high corneal permeability, the cornea could be treated as a single homogeneous barrier (15). Consequently, Scheme 1 (Fig. 5), which does not include the cornea as a compartment, was devised to explain the ocular pharmacokinetic behavior of the lipophilic drugs, ibufenac and ibuprofen. In a previous report by Eller *et al.* (13), ocular drugs with similar physicochemical properties have been adequately explained with the use of Scheme 1.

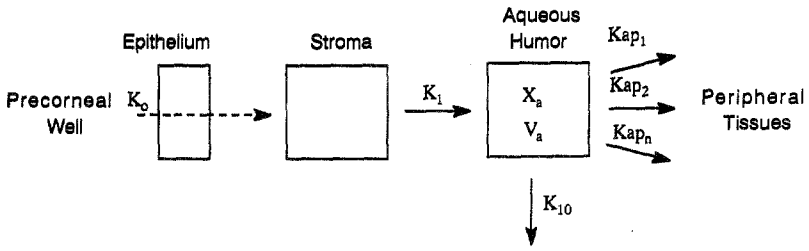
Scheme 2 (Fig. 5) is applied here to describe the ocular pharmacokinetics of R3 and R4. In Scheme 2, the epithelium and endothelium represent significant barriers for drug entering and exiting the cornea with the stroma acting as a separate compartment. The epithelial barrier was justified for these drugs because their log *DC* was significantly less than 0 and because their CPCs were indicative of drugs for which the epithelium is the primary barrier into the cornea (15). At 120 min of infusion, R3 and R4 were assayed for drug content in the stroma. Sixty-four percent of drug resided in the stroma for R3 and 82% for R4.

However, in Fig. 4 the aqueous humor concentration–time curves for R3 and R4 between 120 and 210 min show a less than rapid decline (i.e., shoulder effect) which indicates that the endothelium must be considered a barrier to entry into the anterior chamber. Further justification for the application of Scheme 2 for R3 and R4 came from attempting to fit the data for these drugs to Scheme 1 which was not possible.

In Schemes 1 and 2, X_a is the amount of drug in the aqueous humor and V_a is the physiological volume of the aqueous humor (0.311 ml). K_{10} is the elimination rate constant out of aqueous humor by aqueous humor turnover and uptake by vessels. Although not shown in Scheme 1, K_{el} is a lumped elimination rate constant out of the aqueous humor equaling the summation of K_{10} and $K_{(ap\ 1 \ldots n)}$, the latter representing the first-order transfer rate constants from aqueous humor to peripheral tissues (n). Redistribution from peripheral tissues back into aqueous humor was considered negligible



Scheme 1



Scheme 2

Fig. 5. Compartmental schemes for ibufenac and ibuprofen (Scheme 1) and for R3 and R4 (Scheme 2). K_0 is the zero-order input rate resulting from maintaining a constant concentration of drug on the cornea of anesthetized rabbits. K_{10} is the elimination rate constant out of aqueous humor by aqueous humor turnover and uptake by vessels, and $K_{(ap1...n)}$ represent the first-order transfer rate constants from aqueous humor to peripheral tissues (n).

because of the low peripheral tissue concentrations as well as rates that were relatively slow. In scheme 1, K_0 is the apparent constant (zero-order) input rate from the precorneal area to the aqueous humor, whereas in Scheme 2, K_0 represents the input rate into the stroma and K_1 represents transfer of drug across the endothelium into the aqueous humor.

Noncompartmental Calculations

Equations consistent with the use of Schemes 1 [Eqs. (3)-(6) and (10)] and 2 [Eqs. (6)-(10)] for the topical infusion method of administration are shown below:

$$K_0 = \left[\frac{dC_a}{dt}\right]_I V_a \tag{3}$$

$$K_A = \frac{V_a[dC_a/dt]_I}{C_W V_W} \tag{4}$$

$$Q_e = \frac{K_0 T}{AUC_a} \tag{5}$$

$$MRT_a = \frac{AUMC_a}{AUC_a} - \frac{T}{2} \tag{6}$$

$$K_0 = \left[\frac{dC_c}{dt}\right]_I V_c \tag{7}$$

$$K_A = \frac{V_c[dC_c/dt]_I}{C_W V_W} \tag{8}$$

$$Q_c = \frac{K_0 T}{AUC_c} \tag{9}$$

$$MRT_c = \frac{AUMC_c}{AUC_c} - \frac{T}{2} \tag{10}$$

In Eqs. (3)-(10), C_a and C_c are the concentrations of drug in the aqueous humor and cornea; $(dC_a/dt)_I$ and $(dC_c/dt)_I$ are the initial rates of appearance of drug in the aqueous humor and cornea independent of lag time; V_a and V_c are the volumes of aqueous humor (0.311 ml) and cornea (without epithelial and endothelial layers: 0.039 ml), respectively. K_A is the absorption rate constant for drug either entering the aqueous humor (Scheme 1) or the stroma (Scheme 2) and calculated by estimating the initial slope of the drug concentration in aqueous humor or cornea plotted versus time. This was accomplished by fitting the first 4 or 5 concentration-time points to a polynomial and evaluating either C_a or C_c at $t = 0$ independent of a lag time as described in previous reports (13,22,23) In addition, T is the infusion time period (120 min); V_w is the volume of drug solution in the well remaining in contact with cornea (0.7 ml) over time T; C_W is the concentration of drug in the well during the infusion period; AUC and $AUMC$ are areas to ∞ under the concentration-time curve and the concentration × time-time curve, respectively. The AUC and $AUMC$ values for cornea, aqueous humor, and iris/ciliary body data for ibufenac, ibuprofen, R3, and R4 were obtained from RSTRIP (24) using the trapezoidal rule and used to calculate mean residence times (MRT) for each drug in each tissue.

The volume of distribution at steady state (V_{SS}) for all four drugs was calculated from

$$V_{SS} = \frac{K_0 T \cdot AUMC_a}{AUC_a^2} - \frac{K_0 T^2}{2AUC_a} \tag{11}$$

The ocular pharmacokinetic parameter values obtained from noncompartmental analysis and represented by Eqs. (3)-(11) are listed in Table V. The zero-order input rate constant K_0 calculated from Eqs. (3) and (7) were compared to values that were estimated from (22,23)

$$K_0' = (CPC)A(C_W)60 \tag{12}$$

Equation (12) was valid for ibufenac and ibuprofen since the entire cornea was considered the barrier for entry into the eye for these drugs. K_0' could

Table V. Pharmacokinetic Parameters for Cornea, Aqueous Humor, and Iris/Ciliary Body Determined from Topical Infusion Method Using Noncompartmental Analysis

Parameter	Ibufenac	Ibuprofen	R3	R4
		Cornea		
K_0 (μg/min)[a]	—	—	0.060	0.244
K_A 10^4 (min^{-1})[b]	—	—	0.951	3.86
AUC_c ($\mu g \cdot g^{-1} \cdot min$)[c]	15,831	25,653	6080.3	19,475
$AUMC_c$ ($\mu g \cdot g^{-1} \cdot min^2$)[c]	1,167,289	1,966,046	696,219	2,194,732
MRT_c (min)[d]	13.7	16.6	54.5	52.7
Q_c (μg/min)[e]	—	—	1.18	1.50
		Aqueous humor		
K_0 (μg/min)[f]	0.128	0.272	—	—
K_A 10^4 (min^{-1})[g]	6.08	12.9	—	—
AUC_a ($\mu g \cdot ml^{-1} \cdot min$)[h]	1826	1748.5	875.8	1737.3
$AUMC_a$ ($\mu g \cdot ml^{-1} \cdot min^2$)[h]	154,298	154,321	151,638	280,607
MRT_a (min)[i]	24.5	28.3	113.1	101.5
Q_e (μl/min)[j]	8.39	18.7	—	—
V_{SS} (ml)[k]	0.206	0.527	0.929	1.71
		Iris/ciliary body		
$AUC_{I/CB}$ ($\mu g \cdot g^{-1} \cdot min$)[l]	815.21	1051.7	257.9	956.8
$AUMC_{I/CB}$ ($\mu g \cdot m^{-1} \cdot min^2$)[l]	74,860	88,579	34,478	150,076
$MRT_{I/CB}$ (min)[m]	31.8	24.2	73.7	96.9

[a] K_0 = *In vivo* zero-order input rate constant across the epithelium.
[b] K_A = First-order absorption rate constant across the epithelium.
[c] AUC_c, $AUMC_c$ = Area under the corneal concentration-time curve, area under the corneal concentration × time-time curve, respectively.
[d] MRT_c = Mean residence time for drug disposition in cornea.
[e] Q_c = Apparent corneal clearance.
[f] K_0 = *In vivo* zero-order input rate constant across the whole cornea.
[g] K_A = First-order absorption rate constant across the whole cornea.
[h] AUC_a, $AUMC_a$ = Area under the aqueous humor concentration-time curve, area under the aqueous humor concentration × time-time curve, respectively.
[i] MRT_a = Mean residence time for drug disposition in aqueous humor.
[j] Q_e = Apparent ocular clearance.
[k] V_{SS} = Apparent steady state ocular volume of distribution.
[l] $AUC_{I/CB}$, $AUMC_{I/CB}$ = Area under the iris/ciliary body concentration-time curve, area under the iris/ciliary body concentration × time-time curve, respectively.
[m] $MRT_{I/CB}$ = Mean residence time for drug disposition in iris/ciliary body.

not be used for R3 and R4 because the epithelium represented the barrier for entry into the eye for these drugs, therefore the use of *CPC* was not appropriate. Agreement was relatively good for the comparison of K_0 and K_0' for ibufenac (0.13 vs. 0.19 μg/min) and for ibuprofen (0.27 vs. 0.20 μg/min) and confirmed the use of Scheme 1 to represent the ocular pharmacokinetics of ibufenac and ibuprofen.

The magnitude of K_A for the analog series was directly related to the log *DC* values. The K_A values are relatively small and represent absorption half-lives ($t_{\frac{1}{2}A}$) of 19.00 and 8.96 hr for ibufenac and ibuprofen, respectively. Although not unusually long for the ocular absorption of drugs with similar log *DC* values [e.g., $t_{\frac{1}{2}A} = 8.25$ hr for clonidine (23) and 2.9 hr for pilocarpine (25)], the $t_{\frac{1}{2}A}$ values along with the very short residence time for drugs applied to the eye account for the extremely poor bioavailability of ocular drugs. The hydrophilic drugs, R3 and R4, have $t_{\frac{1}{2}A}$ values that are exceptionally long, 121.5 and 29.9 hr, respectively. However, these values are somewhat less than phenylephrine, 278.4 hr (22), which is also a very hydrophilic drug.

The barrier effect of the endothelium resulted in significantly higher MRT_c for the hydrophilic drugs, R3 and R4, which were approximately 3.5- to 4-fold larger for R3 and R4 compared to ibufenac and ibuprofen. The results confirm the observation that the cornea is acting as a slow-release reservoir. The corneal clearance Q_c for R4 was only slightly higher than R3 which could be attributed to the α-methyl substituent on R4. The a-methyl group had the same effect on the clearance out of the aqueous humor (Q_e) when comparing ibufenac and ibuprofen.

Q_e values for ibufenac and ibuprofen were directly proportional to the lipophilicity of the molecule. Values of 8.39 and 18.7 μl/min were obtained for ibufenac and ibuprofen, respectively. The values are comparable to values determined for clonidine (23) (14.9 μl/min) and by Miller *et al.* (26) for pilocarpine (12 to 13 μl/min) and by Tang-Liu *et al.* (27) for flurbiprofen (14.4 μl/min). Bulk flow of aqueous humor in the rabbit eye is about 1.5% of the volume of the anterior chamber per min (28). If a value of 0.311 ml is used for the volume of aqueous humor, a value of 4.67 μl/min is estimated for aqueous humor clearance by bulk flow. Values of Q_e obtained for the above-mentioned drugs are approximately two to four times greater than bulk flow. This indicates that drug loss is occurring by additional pathways, possibly by uptake into the tissues of the anterior uvea which is highly vascular and/or possibly into the lens or other tissues of distribution without significant reverse diffusion (i.e., redistribution) during the time of the experiment. Clearly then, as a nonsteroidal antiinflammatory agent is structurally modified so that it is more hydrophilic, it is eliminated more slowly from eye tissues. $MRT_{I/CB}$ values (Table V) were higher for R3 and R4

compared to ibufenac and ibuprofen which is consistent with findings for either disposition or elimination pertaining to all tissues for ibufenac, ibuprofen, R3 and R4.

The V_{SS} for ibuprofen, 0.527 ml, is comparable to previously reported values for pilocarpine (29), flurbiprofen (27), and clonidine (23), 0.575, 0.620, and 0.530 ml, respectively. R3 and R4 had higher V_{SS} values, 0.929 and 1.71 ml, respectively. Smaller V_{SS} values (but above 0.311 ml) indicate reduced tissue-binding capability or increased protein binding in aqueous humor. The higher V_{SS} values obtained for R3 and R4 could possibly reflect increased tissue binding. Although iris/ciliary body concentrations are lower for R3 and R4 compared to ibufenac and ibuprofen (see Figs. 3 and 4), other tissues not measured in this study may act as reservoirs. Until more V_{SS} values are determined for ocular drugs, caution must be exercised in interpreting their full meaning. In general, the aqueous humor protein

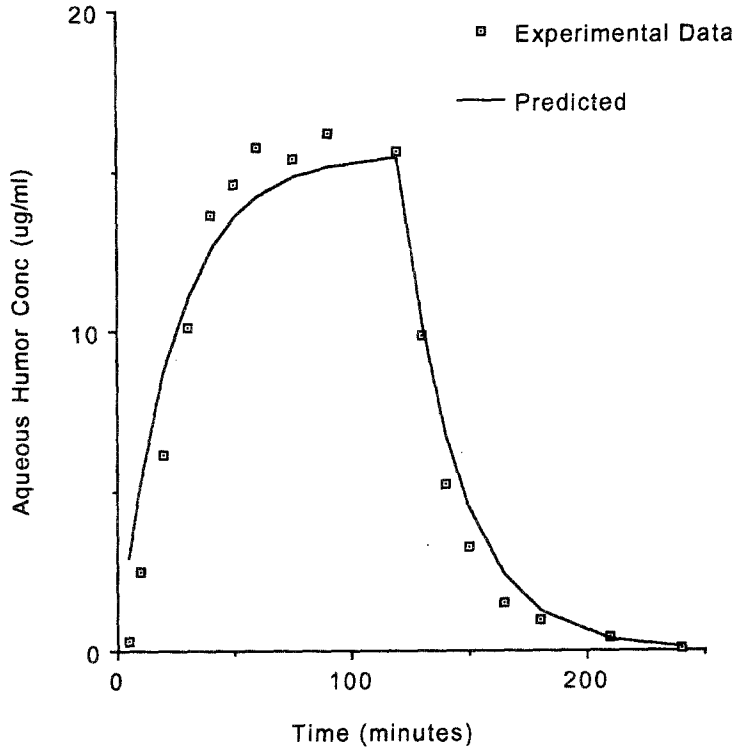


Fig. 6. Computerized fit of ibufenac in aqueous humor following application of a constant concentration of 300 μg/ml to the cornea of anesthetized rabbits for 120 min.

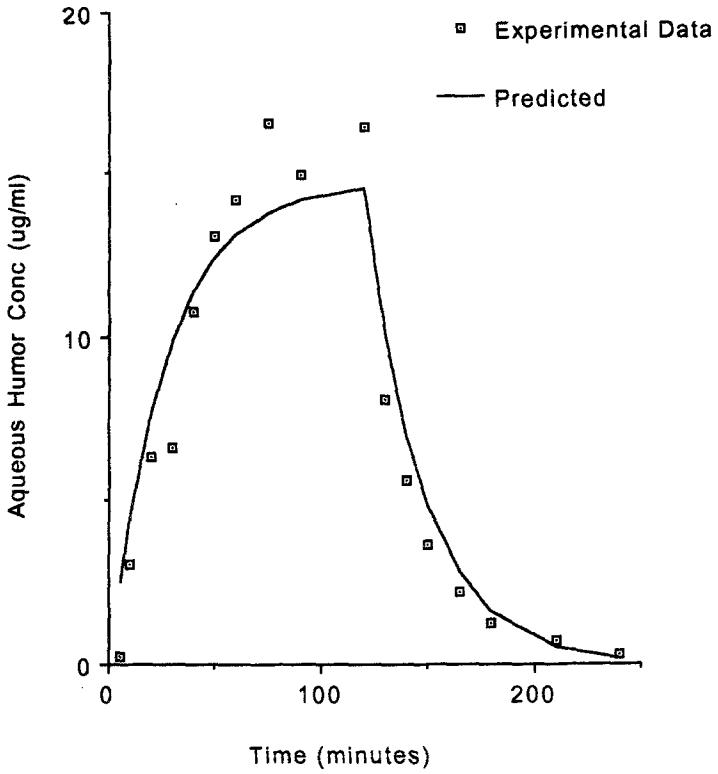


Fig. 7. Computerized fit of ibuprofen in aqueous humor following application of a constant concentration of 300 μg/ml to the cornea of anesthetized rabbits for 120 min.

binding and/or tissue distribution is not as significant in the eye compared to systemic ratios of volume of distribution to the physiological real volume (i.e., plasma volume). For drugs administered systemically, the apparent volume of distribution is usually very much larger than the plasma volume. The V_{SS} for ibufenac, 0.206 ml, is smaller than the physiological volume for aqueous humor (0.311 ml). No plausible explanation can be given for the low value.

Compartmental Analyses

Schemes 1 and 2 provide a basis for the computer fit to the infusion and postinfusion data for cornea and aqueous humor concentrations of each drug. Aqueous humor concentrations of each drug representing infusion and postinfusion phases were fitted simultaneously using BMDP

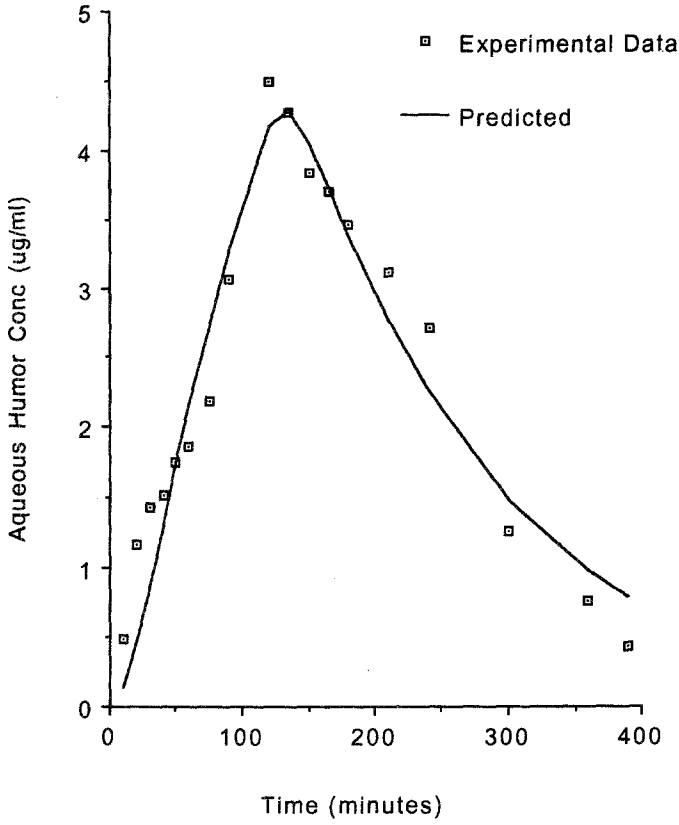


Fig. 8. Computerized fit of R3 [2-(4-hydroxyethoxyphenyl)acetic acid] in aqueous humor following application of a constant concentration of 900 μg/ml to the cornea of anesthetized rabbits for 120 min.

AR (30). V_a was fixed at 0.311 ml, whereas K_0, K_1, and K_{el} were variables. For Scheme 1 the differential equations for infusion and postinfusion are

$$\frac{dC_a}{dt} = K_0 - C_a K_{el} \tag{13}$$

in the infusion phase, and

$$\frac{dC_a}{dt} = -C_{a120} K_{el} \tag{14}$$

in the postinfusion phase.

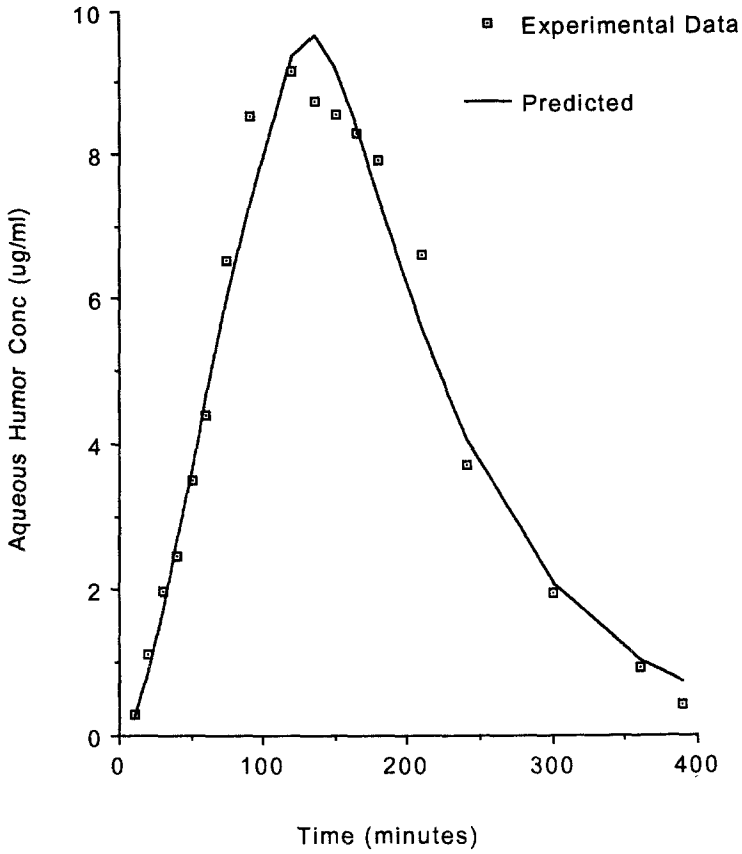


Fig. 9. Computerized fit of R4 [2-(4-hydroxyethoxyphenyl)propionic acid] in aqueous humor following application of a constant concentration of 900 μg/ml to the cornea of anesthetized rabbits for 120 min.

Whereas for Scheme 2 the differential equations are

$$\frac{dC_c}{dt} = K_0 - C_c K_1 \tag{15}$$

and

$$\frac{dC_a}{dt} = C_c K_1 - C_a K_{el} \tag{16}$$

in the infusion phase, and

$$\frac{dC_c}{dt} = -C_{c120} K_1 \tag{17}$$

and

$$\frac{dC_a}{dt} = C_{c120}K_1 - C_{a120}K_{el} \tag{18}$$

in the postinfusion phase.

When integrated, the equations for the infusion and postinfusion phases for Scheme 1 are

$$C_a = \frac{K_0}{V_a K_{el}}(1 - e^{-K_{el}t}) \tag{19}$$

in the infusion phase, and

$$C_a = C_{a120}\, e^{-K_{el}(t-120)} \tag{20}$$

in the postinfusion phase.

Likewise, the integrated equations for the infusion and postinfusion phases for Scheme 2 are

$$C_a = \frac{K_0 K_1}{V_a}\left[\frac{1}{K_1 K_{el}} + \frac{1}{K_1(K_1 - K_{el})}\, e^{-K_1 t} - \frac{1}{K_{el}(K_1 - K_{el})}\, e^{-K_{el}t}\right] \tag{21}$$

in the infusion phase, and

$$C_a = \frac{K_1 C_{c120}}{(K_1 - K_{el})}[e^{-K_{el}(t-120)} - e^{-K_1(t-120)}] + C_{a120}\, e^{-K_{el}(t-120)} \tag{22}$$

in the postinfusion phase.

C_{a120} and C_{c120} represent aqueous humor and corneal concentrations at 120 min when the infusion was terminated. Figures 6–9 represent the experimental and predicted results for aqueous humor drug concentrations for ibufenac, ibuprofen, R3, and R4, respectively. Table VI lists the parameter values K_0, K_1, and K_{el} from the computer fit. The absorption rate constants K_A were much smaller than K_{el} obtained from BMDP AR computer fitting. Without exception, this has been observed for all other drugs studied using the topical infusion technique (13, 15). These results are typical of a 'flip-flop' model (31) and can lead to misinterpretation in assigning the correct value to parameters resulting from nonlinear curve fitting of apparent biexponential profiles when only single-drop instillation is studied. However, when a topical drop is applied, the flip-flop model is not apparent from a method of residual analysis because of the rapid loss from the absorption site which is a consequence primarily of the relatively large drainage rate constant (a parallel process).

Table VI. Ocular Pharmacokinetic Parameter Values for Rate Constants Pertaining to Scheme 1 (Ibufenac and Ibuprofen) and Scheme 2 (R3 and R4) Obtained from BMDP AR Computer Fitting[a]

Compound	K_0 (μg/min)	K_1 (min^{-1})	K_{el} (min^{-1})
Ibufenac	0.200	—	0.0412
Ibuprofen	0.169	—	0.0369
R3	0.0176	0.0651	0.0069
R4	0.053	0.0351	0.0115

[a] K_0 = *In vivo* zero-order input rate constant (schemes 1 and 2); K_1 = Transfer rate constant from stroma to aqueous humor (scheme 2); K_{el} = Lumped elimination rate constant out of aqueous humor (schemes 1 and 2).

The value for K_0 from noncompartmental and classical compartmental calculations vary but are reasonably close. Also, agreement between the fitted and experimental values for each drug concentration in aqueous humor for infusion and postinfusion is good. Therefore, the results for noncompartmental and classical compartmental analyses provide a reasonable explanation for the ocular pharmacokinetics of the lipophilic drugs, ibufenac and ibuprofen, as well as the hydrophilic drugs, R3 and R4. They also indicate that if an active nonsteroidal antiinflammatory drug is too hydrophilic, the initial penetration rate may increase because of increased solubility but its permeability across the cornea and into other ocular tissues may decrease.

R3 and R4 are less active in the topical antiinflammatory models used to evaluate these compounds, however, these models do not differentiate intrinsic potency and activity, the latter of which is a consequence of both pharmacological and pharmacokinetic processes. In order to evaluate intrinsic potency, one must evaluate cyclooxygenase inhibition independent of cellular influences which was not determined in this study. Nevertheless, the pharmacokinetics and ocular irritation potential of R3 and R4 are significantly different from ibufenac and ibuprofen.

As a result of the reduced amphiphilic nature of R3 and R4 compared to ibufenac and ibuprofen, the analogs show less potential to cause tissue irritation, which is a serious concern in the use of opthalmic drugs. Although intrinsic potency may or may not have been altered from the inclusion of a less amphiphilic modification, it is conceivable that R3 and R4 may be too hydrophilic to rapidly cross biological membranes. This is critical to NSAIDs because in addition to absorption they must also enter cells in a sufficient concentration to significantly inhibit cyclooxygenase, an intracellular enzyme. Therefore, the design of new NSAIDs must address cell

penetrability as well as absorption and antiinflammatory activity if adequate therapy is to be achieved.

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