In Vitro Evaluation of Acyloxyalkyl Esters as Dermal Prodrugs of Ketoprofen and Naproxen

JARKKO RAUTIO,*,† HANNU TAIPALE,† JUKKA GYNTHER,† JOUKO VEPSALAINEN,‡ TAPIO NEVALAINEN,† AND TOMI JARVINEN†

Contribution from *Department of Pharmaceutical Chemistry, and Department of Chemistry, University of Kuopio, P.O. Box 1627 FIN-70211, Kuopio, Finland.*

Received December 9, 1997. Final revised manuscript received July 21, 1998.
Accepted for publication July 24, 1998.

Abstract □ A series of acyloxyalkyl esters of ketoprofen and naproxen were synthesized and investigated as topical prodrugs with the aim of improving the dermal delivery of the drugs. In addition, some hydroxyalkyl esters of ketoprofen and naproxen were synthesized as possible intermediates of acyloxyalkyl prodrugs. All of the prodrugs were more lipophilic than their parent molecules, as evaluated by drug partitioning between 1-octanol and phosphate buffer at pH 7.4 (log P_{app}). However, their solubilities in aqueous solutions decreased markedly compared with the parent molecules. The prodrugs were stable toward chemical hydrolysis in aqueous solutions (pH 7.4), but were hydrolyzed to the parent drug both in 80% human serum and in human skin homogenate, with half-lives ranging from 4 to 137 min and from 13 to 403 min, respectively. The abilities of the selected naproxen acyloxyalkyl prodrugs to deliver naproxen through excised human skin were evaluated. Generally, the prodrugs showed similar dermal delivery as the parent drug through cadaver skin. In the present series of lipophilic prodrugs of naproxen, the prodrug with the highest aqueous solubility was the most effective prodrug to deliver naproxen through the skin.

Introduction

Ketoprofen and naproxen are nonsteroidal antiinflammatory drugs (NSAIDs) and are used through the oral or suppository routes for the treatment of pain and inflammation. Given orally, gastrointestinal (GI) side-effects constitute the most frequent of all the adverse reactions of NSAIDs.[1] Therefore, percutaneous administration of NSAIDs, like ketoprofen and naproxen, has been studied to minimize GI side-effects and other possible systemic side-effects due to high plasma peak levels after oral administration.[2−4] Recent studies have shown that GI side-effects of NSAIDs are, at least partly, due to inhibition of the COX-1 enzyme, and thus it may not be possible to eliminate GI side-effects of NSAIDs by percutaneous administration.[5] However, a topically administered drug would be more suitable for treatment of local inflammatory and pain processes because of higher local drug concentration.[6]

An essential prerequisite for the development of a dermal drug delivery system is that the drug be capable of penetrating the skin at a sufficiently high rate to obtain the desired pharmacologic activity. Because most drugs such as ketoprofen and naproxen show unsuitable physicochemical properties, various strategies to aid skin penetration have been studied. Penetration enhancers have been extensively used to achieve this goal. They are chemical compounds that are themselves pharmacologically inactive, but can partition into skin, and interact with the constituents of the superficial skin layer and reduce the resistance of skin to drug diffusion.[7] The selection of formulations including different vehicle compositions is one strategy because properties of the vehicle lead to changes in drug solubility, diffusion, and partition into skin and, therefore, influence the permeability of a drug.[8]

The prodrug approach represents an alternative method of enhancing the skin permeability of drugs. The prodrugs are bioreversible pharmacologically inactive derivatives of a drug molecule that require a chemical or enzymatic transformation to release the active parent drug in situ.[9] Because the epidermis is relatively rich in nonspecific esterases and other enzymatic activity,[10,11] the prodrug approach has been increasingly used to improve delivery of a drug through the skin and/or to localize drug action within the skin.[12−15] However, high prodrug concentration in skin may lead to enzyme saturation kinetics and as a result, limited conversion of prodrug to the parent drug. A recent study with ketorolac acid esters suggested that a large fraction of intact ester may transport into the cutaneous microcirculation because of low biotransformation in human epidermis, and convert to the parent drug by the serum esterases.[15]

A successful dermal prodrug of ketoprofen and/or naproxen should exhibit optimum lipophilicity (partition coefficient) and should be stable toward chemical degradation prior to hydrolysis within the skin by enzymes. The temporary masking of the carboxylic group of ketoprofen and naproxen via simple esterification has been proposed as a promising means of reducing GI irritation[16] and it is also a useful means of modifying the lipophilicity of parent molecules to optimize partitioning into the skin and to maximize percutaneous penetration.[17,18] Simple naproxen esters release naproxen very slowly in human serum and skin−serum homogenate, but a few prodrug esters penetrate the skin markedly better than naproxen. However, conclusions cannot be drawn because drug penetration was calculated as the sum of the prodrug and naproxen.[18]

In the present study, a number of acyloxyalkyl esters of ketoprofen and naproxen have been synthesized for topical drug delivery and their aqueous solubilities, lipophilicities, and hydrolysis rates in buffer, human serum, and skin homogenate were investigated. The permeation of selected naproxen prodrugs across the excised human skin was studied in vitro. In addition, some hydroxyalkyl ester derivatives of ketoprofen and naproxen were synthesized and investigated as possible intermediates of acyloxyalkyl prodrugs.

* Author to whom all correspondence should be sent. Telephone: 358-17-163445. Fax.: 358-17-162456. E-mail: Jarkko.Rautio@uku.fi.
† Department of Pharmaceutical Sciences.
‡ Department of Chemistry.

Experimental Section

Chemicals—Naproxen was kindly donated by Orion Pharma (Espoo, Finland) and ketoprofen was purchased from Sigma (St. Louis, MO). 2-Bromoethanol, 3-bromo-1-propanol, bromomethyl acetate, 2-bromoethyl acetate, 3-chloropropyl acetate, 4-bromobutyl acetate, chloromethyl pivalate, and isopropyl myristate were purchased from Aldrich (Steinheim, Germany). 4-Bromo-1-butanol was synthesized according to previously described method.[19] Acetonitrile used in the HPLC procedures was of HPLC grade and was purchased from Rathburn (Walkerburn, UK), and the bulk solvents bought were from Merck (Darmstadt, Germany). Bovine albumin was purchased from Pierce (Rockford, IL).

General Methods of Synthesis of Prodrugs—Ketoprofen (**I**–**VIII**) and naproxen (**IX**–**XVI**) prodrugs (Figure 1) were synthesized according to the general procedure described for nalidixic acid esters.[20] Briefly, a mixture of either ketoprofen sodium salt or naproxen sodium salt and one equivalent of appropriate hydroxyalkyl or acyloxyalkyl reagent in *N*,*N*-dimethylformamide (10 mL) was stirred at 60 °C for 24 h. Water (50 mL) was added to the reaction mixture, and the mixture was extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with a 5% aqueous solution of sodium carbonate (2 × 25 mL) and water (2 × 25 mL), dried over anhydrous calcium sulfate, and evaporated under reduced pressure. The residue obtained was purified by the semipreparative HPLC method described later (method 3). The abbreviations of ^{1}H chemical shifts are as following: tm is triplet of multiplets; ddd is doublet of doublets of doublets; dm is doublet of multiplets; bs is broad singlet; qui is quinted; td is triplet of doublets.

*Ketoprofen Hydroxyethyl Ester (**I**)*—82% yield; ^{1}H NMR ($CDCl_3$, δ): 7.80 (2H, m), 7.79 (1H, tm), 7.67 (1H, ddd), 7.60 (1H, m), 7.55 (1H, dm), 7.49 (2H, m), 7.44 (1H, tm), 4.23 (2H, m), 3.85 (1H, q), 3.78 (2H, bs), 1.98 (OH, bs), 1.56 (3H, d); high-resolution mass spectrometry (HRMS): data not available.

*Ketoprofen Hydroxypropyl Ester (**II**)*—48% yield; ^{1}H NMR ($CDCl_3$, δ): 4.25 (2H, m), 3.58 (2H, m), 1.90 (OH, bs), 1.83 (2H, qui); HRMS: calcd for $C_{19}H_{20}O_5$: 312.136; found, 312.139.

*Ketoprofen Hydroxybutyl Ester (**III**)*—22% yield; ^{1}H NMR ($CDCl_3$, δ): 4.12 (2H, m), 3.58 (2H, t), 2.01 (OH, bs), 1.70 (2H, m), 1.68 (2H, m); HRMS: calcd for $C_{20}H_{22}O_4$: 326.152; found, 326.148.

*Ketoprofen Acetyloxymethyl Ester (**IV**)*—64% yield; ^{1}H NMR ($CDCl_3$, δ): 7.79 (2H, m), 7.74 (1H, tm), 7.68 (1H, ddd), 7.59 (1H, m), 7.53 (1H, dm), 7.49 (2H, m), 7.44 (1H, td), 5.74 (1H, d)/5.72 (1H, d), 3.84 (1H, q), 2.04 (3H, s), 1.55 (3H, d); HRMS: calcd for $C_{19}H_{18}O_5$: 326.115; found, 326.114.

*Ketoprofen Acetyloxyethyl Ester (**V**)*—49% yield; ^{1}H NMR ($CDCl_3$, δ): 4.28 (2H, m), 4.22 (2H, m), 1.97 (3H, s); HRMS: calcd for $C_{20}H_{20}O_5$: 340.131; found, 340.131.

*Ketoprofen Acetyloxypropyl Ester (**VI**)*—90% yield; ^{1}H NMR ($CDCl_3$, δ): 4.16 (2H, m), 4.05 (2H, m), 2.02 (3H, s), 1.92 (2H, m); HRMS: calcd for $C_{21}H_{22}O_5$: 354.147; found, 354.149.

*Ketoprofen Acetyloxybutyl Ester (**VII**)*—82% yield; ^{1}H NMR ($CDCl_3$, δ): 4.11 (2H, t), 4.02 (2H, t), 2.02 (3H, s), 1.66 (2H, m), 1.61 (2H, m); HRMS: calcd for $C_{22}H_{24}O_5$: 368.162; found, 368.164.

*Ketoprofen Pivaloyloxymethyl Ester (**VIII**)*—51% yield; ^{1}H NMR ($CDCl_3$, δ): 5.76 (1H, d), 5.73 (1H, d), 1.11 (9H, s); HRMS: calcd for $C_{22}H_{24}O_5$: 368.162; found, 368.162.

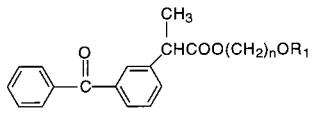
*Naproxen Hydroxyethyl Ester (**IX**)*—96% yield; ^{1}H NMR ($CDCl_3$, δ): 7.65 (2H, d), 7.63 (1H, m), 7.38 (1H, dd), 7.11 (1H, dd), 7.07 (1H, d), 4.14 (2H, m), 3.86 (1H, q), 3.84 (3H, s), 3.66 (2H, t), 2.60 (OH, bs), 1.55 (3H, d); HRMS: calcd for $C_{16}H_{18}O_4$: 274.121; found, 274.120.

*Naproxen Hydroxypropyl Ester (**X**)*—87% yield; ^{1}H NMR ($CDCl_3$, δ): 4.19 (2H, m), 3.50 (2H, t), 2.25 (OH, bs), 1.75 (2H, qui); HRMS: calcd for $C_{17}H_{20}O_4$: 288.136; found, 288.135.

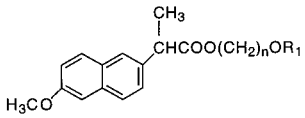
*Naproxen Hydroxybutyl Ester (**XI**)*—21% yield; ^{1}H NMR ($CDCl_3$, δ): 4.10 (2H, t), 3.53 (2H, t), 1.80 (OH, bs), 1.64 (2H, m), 1.50 (2H, m); HRMS: calcd for $C_{18}H_{22}O_4$: 302.152; found, 302.151.

*Naproxen Acetyloxymethyl Ester (**XII**)*—87% yield; ^{1}H NMR ($CDCl_3$, δ): 7.70 (1H, m), 7.69 (1H, d), 7.69 (1H, d), 7.37 (1H, dd), 7.14 (1H, dd), 7.10 (1H, d), 5.73 (1H, d), 5.70 (1H, d), 3.89 (1H, s), 3.88 (1H, q), 1.97 (3H, s), 1.58 (3H, q); HRMS: calcd for $C_{17}H_{18}O_5$: 302.115; found, 302.115.

*Naproxen Acetyloxyethyl Ester (**XIII**)*—93% yield; ^{1}H NMR ($CDCl_3$, δ): 4.27 (2H, m), 4.21 (2H, m), 1.91 (3H, s); HRMS: calcd for $C_{18}H_{20}O_5$: 316.131; found, 316.133.



		n	R_1
I	Ketoprofen hydroxyethyl ester	2	-H
II	Ketoprofen hydroxypropyl ester	3	-H
III	Ketoprofen hydroxybutyl ester	4	-H
IV	Ketoprofen acetyloxymethyl ester	1	-$COCH_3$
V	Ketoprofen acetyloxyethyl ester	2	-$COCH_3$
VI	Ketoprofen acetyloxypropyl ester	3	-$COCH_3$
VII	Ketoprofen acetyloxybutyl ester	4	-$COCH_3$
VIII	Ketoprofen pivaloyloxymethyl ester	1	-$COC(CH_3)_3$



		n	R_1
IX	Naproxen hydroxyethyl ester	2	-H
X	Naproxen hydroxypropyl ester	3	-H
XI	Naproxen hydroxybutyl ester	4	-H
XII	Naproxen acetyloxymethyl ester	1	-$COCH_3$
XIII	Naproxen acetyloxyethyl ester	2	-$COCH_3$
XIV	Naproxen acetyloxypropyl ester	3	-$COCH_3$
XV	Naproxen acetyloxybutyl ester	4	-$COCH_3$
XVI	Naproxen pivaloyloxymethyl ester	1	-$COC(CH_3)_3$

Figure 1—Chemical structures of ketoprofen and naproxen prodrugs (**IV**–**VIII** and **XII**–**XVI**) and their intermediates (**I**–**III** and **IX**–**XI**).

*Naproxen Acetyloxypropyl Ester (**XIV**)*—85% yield; ^{1}H NMR ($CDCl_3$, δ): 4.15 (2H, m), 4.04 (2H, m), 1.91 (3H, s), 1.89 (2H, m); HRMS: calcd for $C_{19}H_{22}O_5$: 330.147; found, 330.145.

*Naproxen Acetyloxybutyl Ester (**XV**)*—73% yield; ^{1}H NMR ($CDCl_3$, δ): 4.09 (2H, t), 3.98 (2H, t), 1.98 (3H, s), 1.62 (2H, m), 1.57 (2H, m); HRMS: calcd for $C_{20}H_{24}O_5$: 344.162; found, 344.162.

*Naproxen Pivaloyloxymethyl Ester (**XVI**)*—65% yield; ^{1}H NMR ($CDCl_3$, δ): 5.76 (1H, d), 5.73 (1H, d), 1.05 (9H, s); HRMS: calcd for $C_{20}H_{24}O_5$: 344.162; found, 344.163.

NMR Spectroscopy—^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 WB operating at 400.1 and 100.6 MHz, respectively, using tetramethylsilane (TMS) as a reference and $CDCl_3$ as the solvent. The spectra were acquired using 32 kW data points with zero filling to a point resolution of >0.1 Hz. ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{1}H nuclear Overhauser enhancement spectroscopy (NOESY) spectra (mixing time 0.7 s) were measured using 256*512 matrixes and ^{1}H–^{13}C correlated spectra were measured using 128*2048 matrixes with zero filling. On request, the ^{13}C chemical shifts are available from authors.

Electron-Impact Ionization Mass Spectrometry—Electron impact (EI) mass spectra of the prodrugs were recorded on a VG 70–250SE magnetic sector mass spectrometer (VG Analytical, Manchester, UK). The resolution of the instrument was adjusted to 10.000. The electron energy was 70 eV, the ionization current was 500 μA, and the ion source temperature was 200 °C. Samples were introduced to the mass spectrometer in a glass sample holder with a direct insertion probe. The probe temperature was raised from 50 to 300 °C in 5 min.

Liquid Chromatography—*Method 1*—The analytical HPLC system for determination of physicochemical properties of prodrugs consisted of a Beckman model 116 pump with a model 166 UV detector, a Marathon automatic sample injector, and a Osborne MD 700 computer. The column was a Kromasil 100-C8 (150 × 4.6 mm, 5 μm) reversed-phase column. A mixed mobile phase of acetonitrile, water, and acetic acid (55:44:1, v/v) at a flow rate of

1.2 mL/min was used. The column effluent was monitored at 254 nm for ketoprofen prodrugs and at 230 nm for naproxen prodrugs.

Method 2—The analytical HPLC system for determination of drug in skin permeation samples consisted a Merck Hitachi L-6200A intelligent pump, Hewlett-Packard HP1046A programmable fluorescence detector (exitation 226 nm; emission 368 nm), a Merck Hitachi D-6000A interface module, a Merck Hitachi AS-2000 autosampler, and a Merck LaChrom column oven L-7350. Separations were performed with a Purospher RP-18 endcapped reversed-phase column (125 × 4.0 mm, 5 μm). A mobile phase mixture of methanol and a 0.02 M phosphate buffer solution of pH 5.5 at a flow rate of 1.2 mL/min were used. The proportion of methanol in the mobile phase was increased linearly from 55 to 80% over 10 min, maintained for 3 min, then returned to the initial conditions over 4 min. Quantitation of the compounds was made from measurements of the peak areas in relation to those of standards chromatographed under the same conditions.

Method 3—The semipreparative HPLC system consisted of a Shimadzu model LC-6A with a model SPD-6A detector (350 nm), a Rheodyne 480 injector module with a 2-mL loop, and a Shimadzu model SCL-6B system controller. The column was a Kromasil KR100−5-C8 (250 × 10 mm, 5 μm) and the isocratic solvent system was acetonitrile and water (55:45, v/v). The flow rate was 5.0 mL/min.

Determination of Solubilities—The solubilities of ketoprofen, naproxen, and their acyloxyalkyl prodrugs (**IV**−**VIII**, **XII**−**XVI**) were determined in phosphate buffer at pH 5.0 and 7.4, and the solubilities of the prodrugs (**VII**, **XII**−**XVI**) only were determined in isopropyl myristate (IPM). These solubilities were measured at room temperature by placing excess amounts of each compound in 1−5 mL of one of these solvents. The slurries in phosphate buffers were placed in an ultrasonic bath for 3 min and then stirred for 3−4 h and filtered (Millipore 0.45 μm). The filter did not absorb compounds after its saturation with 1 mL of solution. Triplicate determinations were made for each compound. The slurries in IPM were stirred for 1 h and, after filtration, the solution was diluted with HPLC eluent. Determinations were made once. The concentrations of the compounds in their saturated solutions were determined by the HPLC method described earlier (method 1). The hydrolysis of esters were negligible during the solubility studies.

Determination of Partition Coefficient—The apparent partition coefficients (P_{app}) of ketoprofen and naproxen and their acyloxyalkyl prodrugs (**IV**−**VIII**, **XII**−**XVI**) were determined at room temperature in a 1-octanol−phosphate buffer (pH 7.4) system. Before use, the 1-octanol was saturated with phosphate buffer for 24 h by stirring vigorously. A known concentration of prodrug in pH 7.4 phosphate buffer (0.16 M) was shaken for 60 min with a suitable volume of 1-octanol to achieve equilibrium. After shaking, the phases were separated by centrifugation at 4000 rpm for 10 min. All experiments were performed in triplicate. The concentrations of the compounds in the buffer phase before and after partitioning were determined by the HPLC method described earlier (method 1).

Hydrolysis in Aqueous Solution—The rates of chemical hydrolysis of prodrug derivatives (**I**−**XIV**) were determined in 0.16 M phosphate buffer (pH 7.4, ionic strength 0.5) at 37 °C. Solutions of prodrugs were prepared by adding an appropriate amount of compound to 2.5 mL ethanol (prodrugs **IV**−**VII**, **XII**−**XV**) or to 5.0 mL ethanol (prodrugs **VIII** and **XVI**). Ethanol was used to effect solution. After vortexing and sonicating the solutions for 10 min, preheated phosphate buffer was added (25 mL). The solutions were kept in a water bath at 37 °C, and, at appropriate intervals, samples were taken and were analyzed for remaining prodrug and any ketoprofen and/or naproxen formed, by using the HPLC method described earlier (method 1). A single determination was made for each prodrug.

Hydrolysis in Human Serum—The rate of hydrolysis for ketoprofen and naproxen prodrugs (**I**−**XVI**) was determined in human serum (Institute of Public Health, University of Kuopio) diluted to 80% with 0.16 M phosphate buffer of pH 7.4 at 37 °C. The prodrugs (~5 μmol) were added to phosphate buffer. After vortexing, preheated human serum was added. The solutions were kept in a water bath at 37 °C. At suitable intervals, 1.0-mL samples of serum/buffer mixture were withdrawn and added to 2.0 mL of ethanol to precipitate protein from the serum. After mixing and centrifugation, the resulting clear supernatant was analyzed for remaining prodrug and ketoprofen and/or naproxen by HPLC (method 1). Pseudo-first-order half-times ($t_{1/2}$) for the hydrolysis of the prodrugs were calculated from the linear slopes of plots of the logarithm of residual prodrugs against time. The pseudo-first-order times at which 50% of total parent compound had been formed ($t_{50\%}$) were determined from the linear slopes of plots of the logarithm of unformed parent compound (log(parent compound_{max} − parent compound_t)) against time.[21] Duplicate determinations were made for each prodrug. The protein concentration in the serum (47 mg/mL) was determined by a dye-binding assay, with bovine serum albumin (BSA) as the standard.[22]

Hydrolysis in Human Skin Homogenate—The rates of hydrolysis of ketoprofen and naproxen prodrugs (**IV**−**VIII**, **XII**−**XVI**) in human skin homogenate (see Skin Preparation section) were investigated by placing ~5 μmol of prodrugs in 10.0 mL of phosphate buffer (0.05 M, pH 7.4). The solutions were either filtered (**IV**−**XIII**) or centrifuged (**XIV**−**XV**), and 1.0 mL of preheated skin homogenate (37 °C) was added to 9.0 mL of clear prodrug solution. The solutions were placed in a water bath and, at suitable intervals, 0.5-mL samples were withdrawn and each mixed with 1.0 mL of ethanol. The mixture was centrifugated and analyzed by HPLC using the method described earlier (method 1). The $t_{1/2}$ values for the hydrolysis of the prodrugs were calculated from the linear slopes of plots of the logarithm of residual prodrug concentration versus time. The formation of parent compound ($t_{50\%}$) was determined by the method described earlier.[21] A single determination was made for each prodrug.

Skin Preparation—Samples of human skin, not >5 days old, were obtained from adult human cadavers. The fresh skin was immersed in water at 60 °C for 2 min, after which stratum corneum and epidermis were removed from the dermis.[23] The skin specimens were placed on a Teflon plate, dried for 2−4 days at room temperature, and frozen.

For the skin homogenate, a sufficient amount of dried human skin was homogenized (Ystral 10/25, dispensing tool 10G, Dottingen, Germany), in 50 mM phosphate buffer (pH 7.4) at 22 000 rpm, to obtain a final concentration of 360 mg/mL. The supernatant was separated after centrifugation at 14000*g* (Eppendorf 5415C, Hinz GmbH., Hamburg, Germany), and lipid drops were removed from the surface of the clear mixture and then stored at −20 °C until use. The protein concentration in the skin homogenate was determined to be 560 μg/mL by a dye-binding assay, with BSA as the standard.[22]

In Vitro Skin Permeation Study—In permeation studies, the dried and frozen skin specimens were thawed, rehydrated, and mounted in Franz-type diffusion cells (PermeGear, Inc., Riegelsville, PA) with the stratum corneum facing the donor chamber. The surface area of the diffusion cell was 0.71 cm^2. The receptor chamber was filled with isotonic phosphate buffer (0.05 M, pH 7.4) containing 0.02% sodium azide as a preservative. Samples of naproxen and its prodrugs (5.0 mM) were applied as suspensions in this buffer solution. The receptor phase was stirred magnetically and kept at a constant temperature of 37 °C with a circulating water bath throughout the study. Samples were taken from the receptor compartment at appropriate time intervals, with replacement with fresh buffer solution. The drug concentrations were assayed by HPLC as described earlier (method 2) and corrected for dilution attributable to the sampling procedure. The skin flux values for rate of delivery of naproxen and its prodrugs **XIII**, **XIV**, and **XVI** were determined from Fick's law of diffusion (eq 1):

$$J_{ss} = V/A(\mathrm{d}C/\mathrm{d}t) \quad (1)$$

where J_{ss} is the steady-state skin flux (nmol/cm^2/h), V is the receptor volume (mL), A is the surface area of the diffusion cell (cm^2), C is the receptor concentration (nmol/mL), and t is time (h).

The steady-state skin flux was determined from the slope of the linear portion of the cumulative amounts (in nmol) of the parent drugs, intermediates, and intact prodrugs measured in the receptor phase versus time plot. The lag time (t_{lag}) was determined by extrapolating the linear portion of the curve to the abscissa. The experiments were performed at least in triplicate.

Results and Discussion

Synthesis and Characterization of Prodrugs—The acyloxyalkyl prodrugs of ketoprofen (**IV**−**VIII**) and naproxen (**XII**−**XVI**), found in Figure 1, have not been reported

Table 1—Apparent Partition Coefficient (mean ± SD; $n = 3$), Aqueous Solubility (mean ± SD; $n = 3$), and Lipid Solubility (One Determination) of Ketoprofen or Naproxen and their Various Acyloxyalkyl Prodrugs

compound	log P_{app}[a]	aqueous solubility (μM) pH 7.4	aqueous solubility (μM) pH 5.0	lipid solubility (mM) IPM[b]
ketoprofen	−0.2 ± 0.01	1 400 000	3250	46 ± 3
IV	3.4 ± 0.03	72 ± 6	64 ± 3	—[c]
V	3.1 ± 0.01	89 ± 4	111 ± 2	—[c]
VI	3.6 ± 0.1	47 ± 6	56 ± 1	—[c]
VII	3.6 ± 0.1	22 ± 3	18 ± 2	>280[d]
VIII	3.5 ± 0.1	2 ± 0.1	2 ± 0.1	—[c]
naproxen	0.3 ± 0.03	102 000	400	22 ± 3
XII	3.4 ± 0.1	8 ± 1	7 ± 2	>90
XIII	3.5 ± 0.03	60 ± 3	52 ± 5	>300[d]
XIV	3.3 ± 0.1	4 ± 1	3 ± 0.4	—[c]
XV	3.3 ± 0.03	14 ± 1	13 ± 0.5	>320[d]
XVI	2.8 ± 0.04	0.4 ± 0.1	0.3 ± 0.03	>440[d]

[a] P_{app} is an apparent partition coefficient between phosphate buffer (pH 7.4) and 1-octanol at room temperature. [b] Isopropylmyristate. [c] Solubility was not determined because of low yield of prodrug. [d] Solubility study was terminated before the saturated condition because of very high lipid solubility of prodrug.

earlier. The hydroxyalkyl esters **I**−**III** and **IX**−**XI** were synthesized as possible intermediates of acyloxyalkyl prodrugs. The HRMS and NMR investigations verified the structures of the prodrug substances. The purities were determinated by HPLC and NMR and they were >95% (mol %) for each prodrug.

Solubility and Apparent Partition Coefficient—Drug solubility is regarded as a key, but not exclusive, parameter that controls the skin permeation of a drug. One of the main aims in dermal prodrug delivery is to obtain a prodrug that not only exhibits increased lipid solubility but also maintains significant water solubility compared with parent drug.[14,24,25] The log P_{app} and aqueous solubilities of ketoprofen and naproxen acyloxyalkyl prodrugs (**IV**−**VIII**, **XII**−**XVI**) were determined at physiological pH 7.4 and at pH 5.0 due to acidic conditions (pH ∼5)[26] on the outer surface of the skin.

The solubilities of ketoprofen, naproxen, and their prodrugs (**IV**−**VIII**, **XII**−**XVI**) in phosphate buffer at pH 5.0 and pH 7.4, the solubilities of prodrugs (**IV**, **VI**−**X**) in IPM, and the log P_{app} values of the compounds are shown in Table 1.

All the prodrugs are markedly more lipophilic than the parent drugs, as illustrated by the log P_{app} values. Due to the acid character of ketoprofen (pK_a 4.60) and naproxen (pK_a 4.15), they are more water soluble at pH 7.4 than at pH 5.0. However, due to a lack of ionizable groups, the prodrugs showed similar aqueous solubilities at both pH values, but all were much lower than that of their respective parent drugs. The acetyloxyethyl esters (**V** and **XIII**) showed the highest aqueous solubility, whereas pivaloyloxymethyl esters (**VIII** and **XVI**) showed the lowest aqueous solubility.

Chemical Hydrolysis in Aqueous Solution—The degradations of ketoprofen and naproxen prodrugs (**I**−**XIV**) were studied in buffer solution (pH 7.4) at 37 °C. The degradation of each prodrug followed first-order kinetics. The $t_{1/2}$ values for degradation of hydroxyalkyl prodrugs (**I**−**III**, **IX**−**XI**) and acyloxyalkyl prodrugs (**IV**−**VIII**, **XII**−**XVI**) are shown in Tables 2 and 3, respectively.

All hydroxyalkyl prodrugs (**I**−**III**, **IX**−**XI**) were highly resistant to chemical hydrolysis. The $t_{1/2}$s (37 °C) of all compounds were >15 days at pH 7.4 and much longer yet at pH 5.0 (data not shown). It is generally assumed that the hydroxyalkyl esters are intermediates in acyloxyalkyl

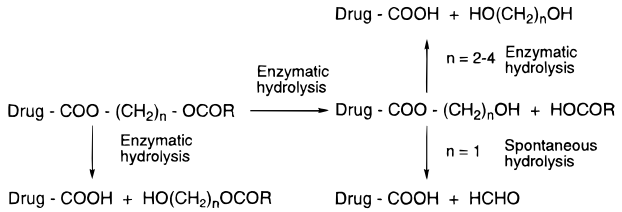
Table 2—Half-Lives ($t_{1/2}$) of Hydroxyalkyl Esters of Ketoprofen (I–III) and Naproxen (IX–XI) in Buffer Solution (pH 7.4) and in Human Serum (pH 7.4) at 37 °C

compound	$t_{1/2}$ (days) phosphate buffer	$t_{1/2}$ (min) 80% human serum
I ketoprofen hydroxyethyl ester	15	124
II ketoprofen hydroxypropyl ester	47	69
III ketoprofen hydroxybutyl ester	38	35
IX naproxen hydroxyethyl ester	25	224
X naproxen hydroxypropyl ester	52	147
XI naproxen hydroxybutyl ester	65	61

Table 3—Rate of Hydrolysis of Ketoprofen Acyloxyalkyl Prodrugs (IV–VIII) and Naproxen Acyloxyalkyl Prodrugs (XII–XVI) In Buffer Solution, In Human Serum, and In Human Skin Homogenate at pH 7.4 and 37 °C

compound	$t_{1/2}$ (h) phosphate buffer	$t_{1/2}$ (min) human serum ($n = 2$)[d]	$t_{50\%}$ (min)[a] human serum ($n = 2$)[d]	$t_{1/2}$(min) human skin homogenate	$t_{50\%}$ (min)[a] human skin homogenate
IV	10	4 (3, 5)	12 (10, 14)	13	13
V	150	25 (24, 26)	29 (28, 29)	344	—[c]
VI	385	44 (42, 45)	61 (59, 62)	403	—[c]
VII	575	11 (10, 12)	35 (32, 37)	66	62
VIII	30	10 (9, 11)	11 (11, 12)	17	26
XII	20	33 (32, 33)	61 (60, 62)	30	50
XIII	400	82 (77, 86)	74 (62, 85)	158	—[c]
XIV	485	137 (136, 137)	149 (145, 153)	139	—[c]
XV	1900	68 (62, 74)	70 (65, 75)	144	—[c]
XVI	100	9 (7, 11)	25 (22, 27)	—[b]	—[b]

[a] $t_{50\%}$ is the time by which 50% of total ketoprofen and/or naproxen has been formed. [b] Not determined because of low aqueous solubility. [c] Not determined because of slow enzymatic hydrolysis of these prodrugs. [d] Ranges are shown in parantheses.



Scheme 1

prodrug metabolism (Scheme 1).[14,27] The present results indicate that hydroxyethyl, -propyl, and -butyl esters of ketoprofen and naproxen do not easily release the parent drug by chemical hydrolysis. The results are in good agreement with earlier reported results for hydroxyalkyl esters of penicillin,[28] mefenamic acid, and diclofenac,[29] all of which exhibited slow hydrolytic degradation in aqueous solutions. On the other hand, the first step in the hydrolyses of the acyloxymethyl prodrug is the hydrolysis of its terminal ester bond with formation of a hydroxymethyl ester, which rapidly hydrolyzes to the parent drug and formaldehyde (Scheme 1). Unfortunately, in this study, no comparison could be made between the respective hydroxymethyl esters and other hydroxyalkyl esters because attempts to synthesize the very unstable hydroxymethyl ester derivatives failed. Acyloxyalkyl prodrugs also proved to be stable in aqueous solutions at pH 7.4. Prodrugs **VI**, **VII**, **XIV**, and **XV** were the most stable prodrugs, whereas acetyloxymethyl esters (**IV** and **XII**) were the most labile prodrugs.

Enzymatic Hydrolysis—An essential prerequisite for the successful topical use of prodrugs is bioconversion of the prodrug in the skin. In the present study, the rates of enzymatic prodrug hydrolysis were determined both in human skin homogenates and in human serum. This

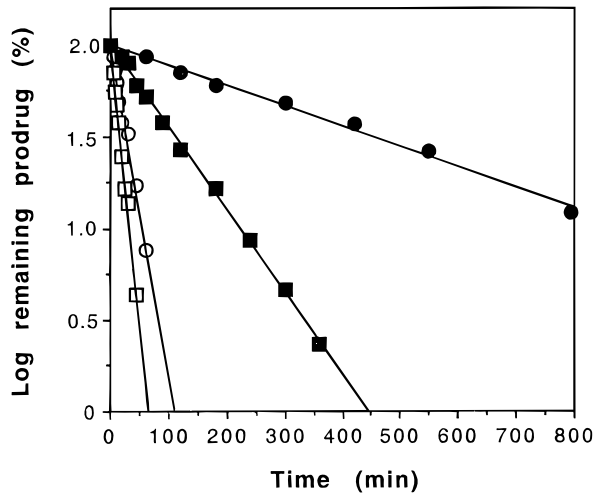


Figure 2—Pseudo-first-order plots for the hydrolysis of ketoprofen prodrug **VIII** (□) and naproxen prodrug **XV** (■) in 80% human serum (pH 7.4) and prodrugs **VIII** (○) and **XV** (●) in human skin homogenate (pH 7.4) at 37 °C.

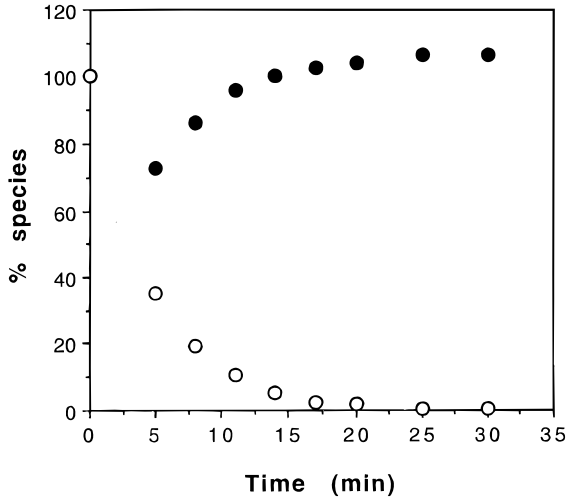


Figure 3—Time courses for ketoprofen acetyloxymethyl ester (**IV**) (○) and ketoprofen (●) during hydrolysis of the prodrug in 80% human serum (pH 7.4) at 37 °C.

method has been applied in assessing bioconversion of prodrugs for dermal delivery.[17,20,30]

Acyloxyalkyl prodrugs (**IV**−**VIII**, **XII**−**XVI**) exhibited pseudo-first-order degradation kinetics for 3−4 half-lives in both serum and skin homogenate (Figure 2). Half-lives ($t_{1/2}$) for the degradation of prodrugs in 80% human serum and in 10% human skin homogenate at 37 °C are displayed in Table 3 with the half-lives for degradation of the prodrugs in pH 7.4 phosphate buffer at 37 °C listed for comparison. Figures 3 and 4 show a typical time course for the degradation of ketoprofen acetoxymethyl ester (IV) and the concurrent formation of ketoprofen in human serum and skin homogenate, respectively.

Hydroxyalkyl ester derivatives of both ketoprofen and naproxen (**I**−**III**, **IX**−**XI**) showed bioconversion half-lives in human serum ranging from 35 to 224 min (Table 2). A longer hydroxyalkyl chain resulted in faster hydrolyses. However, all the ketoprofen and naproxen acyloxyalkyl prodrugs were also highly susceptible to hydrolysis in human serum (Table 3). The half-lives of ketoprofen and naproxen acyloxyalkyl prodrugs ranged from 4 to 44 min and from 9 to 137 min, respectively. The hydrolyses of prodrugs in human skin homogenate were generally slower compared with hydrolyses of the same compounds in serum. Only prodrugs **XII** and **XIV** hydrolyzed similarly

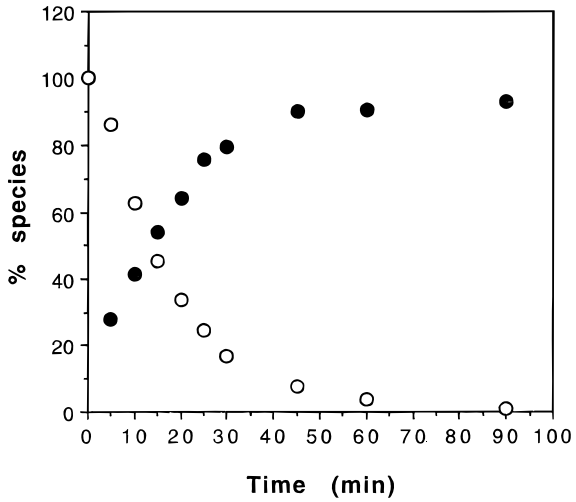


Figure 4—Time courses for ketoprofen acetyloxymethyl ester (**IV**) (○) and ketoprofen (●) during hydrolysis of the prodrug in 10% human skin homogenate (pH 7.4) at 37 °C.

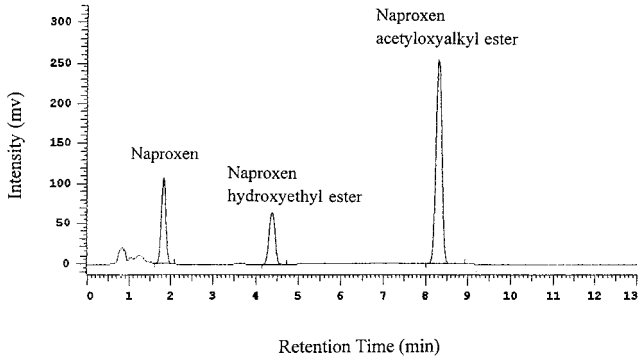


Figure 5—HPLC chromatogram of a sample (30 min) from enzymatic hydrolysis studies of naproxen acetyloxyethyl ester (**XIII**).

in each medium. Previously reported results for the hydrolysis of highly lipophilic naproxen esters showed a similar considerable decline in the rate of hydrolysis of the esters in skin homogenate compared with human serum.[17] However, a true comparison of enzymatic degradation rate of the prodrug between different vehicles is not reasonable because the total esterase activity varies for each.

Table 3 includes $t_{50\%}$ values for ketoprofen and naproxen, which represent the time at which 50% of total parent drug has been formed in human serum and in human skin homogenate.[21] The $t_{50\%}$ values are useful in the evaluation of prodrugs that require many kinetic steps before the parent drug is formed. Therefore, $t_{50\%}$ can be considered a more relevant indicator of the formation of the parent drug than the prodrug degradation rate. In the case of esters **V** and **XIII**, the formations of ketoprofen and naproxen from their acyloxyalkyl esters in human serum were faster than degradation of corresponding hydroxyalkyl esters in human serum or in aqueous solutions. Thus, esters **V** and **XIII** may hydrolyze to the parent drug without formation of the hydroxyalkyl ester as intermediate. However, formation of both ketoprofen/naproxen and corresponding hydroxyalkyl esters was detected in hydrolysis studies on some prodrugs (Figure 5). This result suggests that both hydrolysis mechanisms (via intermediate and direct hydrolysis to the parent drug) are included in the degradation of the acyloxyalkyl prodrugs of ketoprofen and naproxen (Scheme 1). The determined $t_{50\%}$ values for prodrugs **IV**, **VII**, **VIII**, and **XII** in human skin homogenates indicated that formation of the parent drug take place at the same rate as the loss of these prodrugs from solution (Table 3).

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.