Permeability of Ketorolac Acid and Its Ester Analogs (Prodrug) through Human Cadaver Skin

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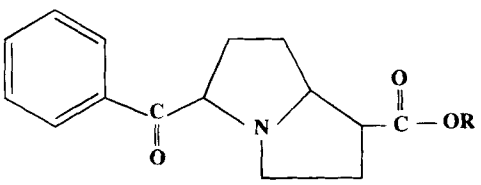
Received May 9, 1994, from the *Syntex Research, 3401 Hillview Avenue, Palo Alto, California 94304.* Accepted for publication August 4, 1994[⊗].

Abstract □ The in vitro skin permeabilities of ketorolac acid (KA), a potent nonsteroidal analgesic, and its two ester analogs as prodrug through human cadaver skin were investigated. The two esters of KA, namely, the ethyl ester (KEE) and [(*N,N*-dimethylamino)carbonyl]methyl ester (KDAE), were selected. The melting temperature of the two esters was significantly lower than that of ketorolac free acid. The partition coefficients ($K_{o/w}$) were 600, 3541, and 124 for KA, KEE, and KDAE, respectively. The enzymatic hydrolysis of KEE and KDAE by human pooled serum at 37 °C was investigated. The esters were hydrolyzed to KA by the serum esterases; the metabolic rate constants were 0.0418 and 0.0148 min^{-1} for KDAE and KEE, respectively. The serum half-life of KDAE was about 3 times shorter than KEE. When split-thickness cadaver skin was incubated with ester solution at 32 °C, the enzymatic hydrolysis of these esters was observed. The metabolic rate in the skin, however, was significantly lower than in the human pooled serum. The skin permeations of KA, KEE, and KDAE through heat-separated epidermis from propylene glycol (PG), PG/glyceryl monocaprylate (GMC) (9:1), and PG/Azone (19:1) vehicle mixtures were evaluated using modified Franz flow-through diffusion cells. The skin fluxes of KA, KEE, and KDAE from PG/GMC (9:1) were 50 ± 10, 15 ± 4, and 57 ± 6 $\mu g/cm^2/h$, respectively. KA was detected in the receiver compartment, albeit to a lesser extent. In conclusion, KDAE appeared to be a better ester prodrug than KEE because it exhibited relatively higher skin flux and faster enzymatic hydrolysis by human serum to liberate the parent drug.

Introduction

Ketorolac tromethamine is a nonsteroidal agent with potent analgesic and moderate anti-inflammatory activity.[1] The permeabilities of ketorolac free acid and ketorolac tromethamine salt through hairless mouse skin and monkey skin were reported.[2] Those investigators clearly demonstrated ketorolac blood levels in the monkey following application of a transdermal solution containing either ketorolac free acid or ketorolac tromethamine salt. Those studies also suggested that the skin permeations of ketorolac free acid and ketorolac tromethamine salt were somewhat similar when the drug was delivered from a vehicle containing potential skin penetration enhancers such as lauric acid and oleic acid.[2]

The use of a chemical penetration enhancer to accelerate the skin permeation of ketorolac may cause local skin irritation leading to erythema and edema of the skin. In some cases, ketorolac acid may not be chemically stable in the presence of certain hydroxylated organic vehicles.[3] In fact, a hydroalcoholic solution of ketorolac acid at a low pH (e.g., pH of less than its pK_a) had a tendency to form a ketorolac acid ester upon long-term storage. As a result, the potency of ketorolac acid in the transdermal solution would significantly decrease during storage even at room temperature. In this respect, the esters of ketorolac acid, as a prodrug approach, may be an alternative way to deliver the drug through human skin to achieve a systemic effect of the drug. Therefore, we evaluated the transdermal delivery of the analogs of ketorolac acid to circumvent the problems encountered with free acid delivery through human skin. The esters of ketorolac acid may have different physicochemical properties (such as partition coefficient and melting point) than those of the parent drug that will facilitate the permeability of esters across the human skin. After penetration through the stratum corneum, a fraction of ketorolac acid ester will be metabolized by viable epidermal and dermal layers to liberate free acid. The remaining ester will reach systemic circulation and will eventually be metabolized to free acid by the plasma esterases. The chemical structures of ketorolac acid and its two ester prodrugs are shown in Figure 1.



Compound	R
Ketorolac acid (KA)	H
Ketorolac acid [(*N,N*-dimethylamino) carbonyl] methyl ester (KDAE)	$CH_2CON(CH_3)_2$
Ketorolac acid ethyl ester (KEE)	C_2H_5

Figure 1—The chemical structure of ketorolac acid and its two esters (prodrug).

The principal objectives of this report are (1) to assess the physicochemical properties of ketorolac acid and its two esters, (2) to evaluate enzymatic hydrolysis of ester by pooled human serum and human cadaver skin, and (3) to compare the skin flux of ketorolac acid and its esters through human cadaver skin from various vehicle formulations.

Experimental Section

Materials—Ketorolac acid (KA), ketorolac acid [(*N,N*-dimethylamino)carbonyl]methyl ester (KDAE), and ketorolac acid ethyl ester (KEE), were synthesized by the Institute of Organic Chemistry at Syntex (Palo Alto, CA). The chemical purity of the esters was determined separately by TLC and HPLC methods; the purity of each ester was found to be greater than 99%. The melting temperature of the free acid and the two esters was determined as described in the USP.[4]

Partition Coefficient Determination—The *n*-octanol/water partition coefficient ($K_{o/w}$) of the drugs were determined by preparing a known amount of the drug solution in 0.01 N HCl (pH = 2.2) for free acid or in water for esters presaturated with *n*-octanol. The drug solution was filtered though 0.22 μm nylon-66 filters and the drug concentration in the aqueous phase was determined by HPLC. About 2 mL of the aqueous drug solution was placed in a vial containing 0.25 mL of octanol presaturated with 0.1 N HCl (free acid) or water

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(ester). The mixture was tumbled for 24 h. The layer was allowed to separate and the pH of the aqueous phase was measured immediately to ensure no change in the pH. After appropriate dilution with acetonitrile, the aqueous layer and *n*-octanol layer were separately assayed by HPLC. The $K_{o/w}$ was determined as the ratio of drug concentration in the octanol phase and in the aqueous phase.

Solubility Determination—Solubilities of KA, KDAE, and KEE in various vehicles were determined by adding an excess of the solid to 5 mL of each test vehicle using a small conical flask of 25 mL capacity. The flask was placed in a water bath maintained at 32 °C and the solution was stirred for 2 days to reach equilibrium. The samples (1.0 mL) were withdrawn and immediately filtered though a syringe connected to a filter unit (Alpha-450, 0.45 μm membrane filter, Gelman Sciences Inc.). The first 25% of the filtrate was discarded to avoid any potential loss of the drug because of adsorption by the filter unit, and subsequent filtrate was collected. All the operations were conducted at 32 °C to avoid any potential precipitation of the drug in the vehicle. The filtrate was appropriately diluted with a mobile phase and assayed by HPLC.

Serum Metabolism—Pooled human serum was used for the in vitro metabolism studies. The drug solutions were prepared in 2% propylene glycol (PG)/water to solubilize ketorolac esters in aqueous media. The serum was diluted to 80% with 0.05 M phosphate buffer of pH 7.4. The enzymatic reaction was initiated by adding 2 mL of the drug solution to 15 mL of pooled human serum that was previously equilibrated to 37 °C. A sample (0.5 mL) was withdrawn at intervals (immediately after mixing, and at 0.5, 2, 5, 10, 15, 20, 40, and 60 min) and transferred to test tubes containing 0.5 mL of chilled acetonitrile to arrest the enzymatic reaction instantaneously. The sample was centrifuged at 5000*g* for 5 min at 4 °C to remove the precipitated proteins. The supernatant was transferred to small vials and the samples were assayed by HPLC. A control for each ester was run simultaneously by adding 2 mL of ester solution to 15 mL of phosphate buffer solution to ensure very little or no hydrolysis of ester during the same period of serum metabolism studies.

Skin Incubation—The enzymatic hydrolysis of esters by split-thickness (ca. 300 μm) human cadaver skin was evaluated. Samples of split-thickness skin were removed from the inner thigh of cadaver skin not later than 24–48 h postmortem with the aid of a dermatome. The skin was placed immediately in Ringer solution and the skin sample was shipped on same day to our laboratories. The skins were used immediately as received for metabolism studies or stored at −20 °C for skin permeation experiments. A known area of the skin (5 cm^2) was die-cut using a hole punch and dried with a paper towel to remove any surface water or moisture. The skin samples were weighed immediately; the skins that were similar in weight were selected for the skin incubation studies. A known concentration of ester solution was prepared in 2% PG in water. Two milliliters of ester solution was added to a glass jar containing 15 mL of 1.15% KCl solution and stirred for 2 min to reach equilibrium. A sample (0.5 mL) was withdrawn to determine the initial concentration of each ester. The dried skin sample was then dropped into a glass jar and placed in a water bath maintained at 32 °C. The solution was stirred using a magnetic bar for 3 min, and a 0.5 mL sample was withdrawn and immediately transferred to a small plastic centrifuge tube containing 0.5 mL chilled acetonitrile to arrest enzymatic reaction. The glass jar was capped to prevent any evaporation and the solution was stirred throughout the duration of the incubation experiment. The subsequent samples were withdrawn at time intervals of 0.5, 1, 2, 4, and 24 h. These samples were treated in a similar fashion, as described. The concentration of ester and ketorolac free acid in the solution was assayed by HPLC methods. A control for each ester was run simultaneously by adding 2 mL of ester solution to 15 mL of 1.15% KCl solution to ensure very little or no hydrolysis of ester during the same period of the skin incubation studies.

Skin Permeation—Human cadaver skin was used for the skin permeation studies. The epidermal layers (i.e., stratum corneum and viable epidermis) were separated from the dermis by immersing the split-thickness skin in water at 60 °C for 60 s and then teased off the epidermis from the dermis. The removed epidermal layer was used as such for the skin diffusion studies.

The flow-through Franz diffusion cell (Crown Glass) was used for the skin permeation studies. A detailed description of similar equipment was reported by Addicks et al.[5] Briefly, the Teflon cells were placed in a diffusion cell heater and the temperature was maintained at 32 °C using a circulating water bath (Haake, Buckler Instrument). The effective diffusion area was 0.32 cm^2. The 10% PEG-400/water was used as a receiver fluid to increase the solubility of each ester in the aqueous media. Because these esters were susceptible to aqueous hydrolysis, the stability of each ester in the receptor fluid at 32 °C was studied. The receiver fluid flow rate was 3 mL/h, and the samples were collected in the scintillation vials. The epidermal layer was equilibrated with receiver fluid for at least 30 min before charging the donor compartment with a drug solution. The receptor samples were collected prior to drug loading in the donor compartment and assayed by HPLC for the presence of any interfering peaks in the chromatograph. Two hundred microliters of each formulation (slurries) was used as the donor phase. These slurries, which were equilibrated to saturation by continuous stirring of an excess of the compound in PG or in mixed vehicles for 2 days at 32 °C, were used to maintain a constant permeant concentration in the donor phase. The samples were collected in scintillation vials every 5 h for 35 h. The samples were assayed by HPLC.

Table 1—Physicochemical Properties of Ketorolac Acid and Its Two Esters

parameter	KA	KDAE	KEE
Molecular weight (g/mol)	255	340	283
Melting point (°C)	156	83	69
$K_{o/w}$	600[a]	124	3541
Aqueous solubility at 25 °C (mg/mL)	0.14	0.32	0.018
pK_a	3.5		

[a] Aqueous phase pH = 2.2.

Drug Assay—Ketorolac acid and its two esters were assayed by HPLC using UV detection. The chromatographic resolutions were obtained on a C_{18} reverse-phase column (Spherisorb ODSI, 5 μm, 25 cm × 4.6 mm, Altech). The mobile phase was methanol/water/acetic acid (60:39:1, pH 3.6) and the flow rate was 1 mL/min. The UV detector was set at 314 nm and the injection volume was 50 μL. The peak area of each compound was integrated on a Spectra Physics integrator. A standard calibration curve was constructed by simply plotting peak area versus concentration of drug.

Data Analysis—The skin flux was determined from Fick's law of diffusion:

$$J_s = V/A(dC/dt) \quad (1)$$

where J_s is the steady-state skin flux in μg/cm^2/h, V is the receptor volume in mL, A is the active diffusion area in cm^2, C is the receptor concentration in μg/mL, and t is time. The steady-state skin flux was determined from the slope of the linear portion of the cumulative amount permeated versus time plot. The lag time (T_{lag}) was determined by extrapolating the linear portion of the curve to abscissa.

Results and Discussion

Physicochemical Properties—The physicochemical properties of ketorolac acid and its two ester prodrugs are listed in Table 1. The molecular weights of KA and esters ranged from 255 to 340 g/mol. The melting points of the KDAE and KEE esters were significantly lower than that of ketorolac free acid. The low melting temperature of the esters was possibly because of an absence of intramolecular hydrogen bonding in the solid state; therefore, ketorolac esters were less crystalline than the free acid. The octanol/water partition coefficient ($K_{o/w}$) of ketorolac free acid was determined to be 600 at pH 2.2. The $K_{o/w}$ of KA, however, was dependent on the pH of the aqueous phase. The $K_{o/w}$ of KEE was about 5 times higher than that of the free acid. In contrast, the $K_{o/w}$ of KDAE was about 5 times lower than that of the free acid. This may partly be due to the relatively high hydrophilicity of the acetoamido functional moiety of KDAE as compared to the carboxylic functional group of KA. A similar trend in aqueous solubility was observed. KEE exhibited the lowest aqueous solubility, while the solubility of KDAE was roughly 2 times higher than that of the free acid. The pK_a of KA in water was reported to

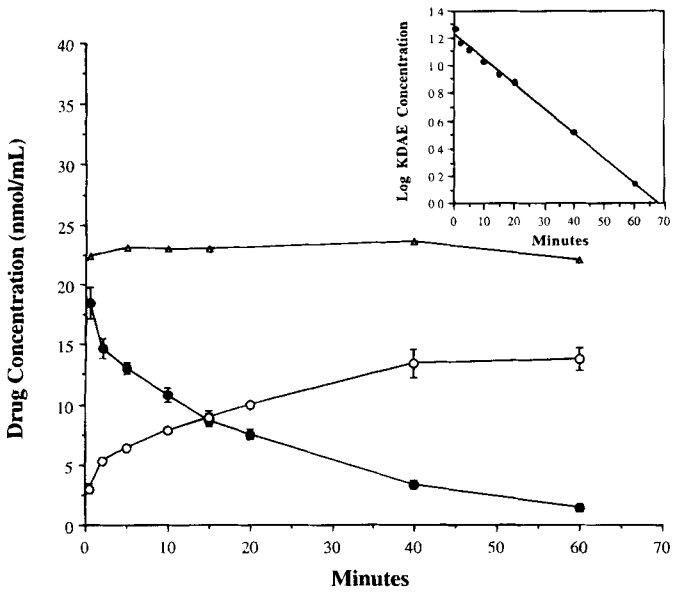

Figure 2—The kinetics of biotransformation of KDAE in pooled human serum at 37 °C. The inset is a log concentration versus time plot showing first-order metabolic degradation kinetics. Key: KDAE (closed circle); KA (open circle); control, KDAE in buffer (open triangle).

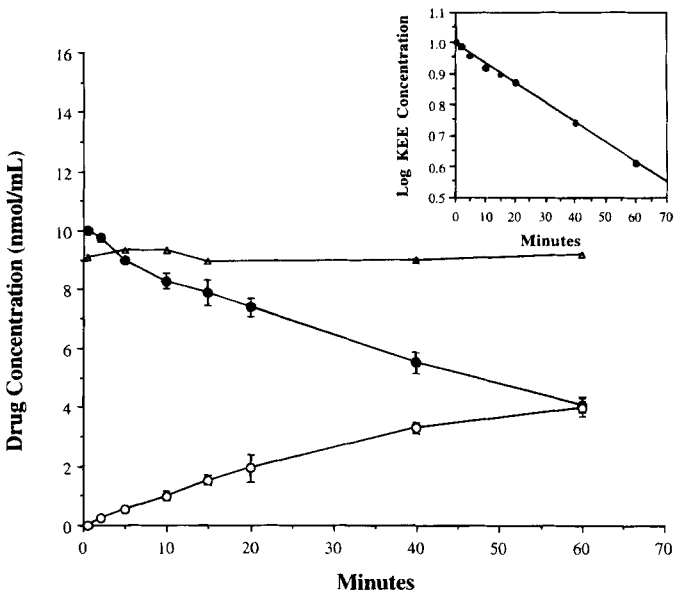

Figure 3—The kinetics of biotransformation of KEE in pooled human serum at 37 °C. The inset is a log concentration versus time plot showing first-order metabolic degradation kinetics. Key: KEE (closed circle); KA (open circle); control, KEE in buffer (open triangle).

be 3.5.[6] The pK_a of KA increased linearly as the volume fraction of alcohol in water was increased (data not shown), consistent with published results for diclofenac.[7] This implied that the ionization of ketorolac free acid would be affected by the presence of hydroxylated organic vehicle in water. In contrast, the esters were nonionizable at all pH values, but at a high pH (say, pH > 10), the esters were susceptible to chemical hydrolysis to liberate free acid.

Serum Hydrolysis of Esters—The enzymatic hydrolysis of the two esters of ketorolac by pooled human serum was evaluated. Because the esters were sparingly soluble in water, especially the ethyl ester (KEE), 2% PG was added to solubilize the ester in an aqueous media so that an appropriate amount of each ester could be readily incorporated in pooled human serum for metabolism studies. Figures 2 and 3 show the kinetics of the serum metabolism of KEE and KDAE. Each plot represents the mean of triplicate samples. Ketorolac free acid was the major metabolite of such enzymatic ester hydrolysis. The concentration of each ester in serum declined exponentially with time, while the concentration of KA in the serum media increased with incubation time. In a control experiment, the ester was incubated with phosphate buffer alone. No significant chemical hydrolysis of either ester in the phosphate buffer solution was noted during the time course of the experiment (Figures 2 and 3). These results strongly suggested that serum esterases were solely responsible for the enzymatic hydrolysis of ester to ketorolac free acid.

The log concentration–time plot indicated that the rate of metabolism followed first-order kinetics (Figures 2 and 3 insets). Table 2 summarizes the rate constant and half-life of the two esters in human serum. The rate constant (k) as determined by the linear regression of the slope (k = slope/2.303) was 0.0418 and 0.0148 min^{-1} for KDAE and KEE, respectively. In other words, the rate of enzymatic hydrolysis of KDAE ($T_{1/2}$ = 16.6 min) was roughly 3 times faster than that of KEE ($T_{1/2}$ = 46.9 min). These results were somewhat contrary to reported results for the serum hydrolysis of carboxylic ester derivatives in which the enzymatic hydrolysis rates increased linearly as the lipophilicity ($K_{o/w}$) of the prodrugs increased.[8] In any case, once the intact ester diffused though the human skin into the bloodstream, the active parent drug would eventually be liberated into the systemic circulation.

Table 2—Serum Metabolism of KDAE and KEE at 37 °C[a]

Ester	R^b	k (min^{-1})	$T_{1/2}$ (min)
KDAE	0.99	0.0418 ± 0.0032	16.6 ± 1.3
KEE	0.99	0.0148 ± 0.0014	46.9 ± 3.4

[a] Each value is the mean ±SD of three independent experiments. [b] R = Correlation coefficient of the linear regression analysis.

Skin Hydrolysis of Esters—It must be noted that the skin incubation experiment with intact split-thickness cadaver skin was preferred over a typical in vitro skin homogenate because the latter techniques might overestimate the enzymatic hydrolysis potential of the skin. In fact, Guzek et al. demonstrated that skin homogenate results might overestimate esterases activity because of increased enzymatic activity and/or decreased capillary removal.[9] Moreover, the diffusion of lipophilic compounds though the stratum corneum was previously demonstrated to be an intercellular lipoidal pathway rather than a transcellular one.[10] Under such circumstances, the skin homogenate experiments would overestimate the enzyme hydrolysis of these esters. Therefore, an intact skin incubation experiment appeared to evaluate more realistically the metabolic fate of a drug in the skin.

Figures 4 and 5 show the enzymatic hydrolysis of KDAE and KEE upon incubation of split-thickness cadaver skin with the ester solution at 32 °C. Each plot represents the average of duplicate samples using thee different skin donors (n = 6). In contrast to serum metabolism studies, a temperature of 32 °C was used for the skin incubation studies to mimic the skin surface temperature. Interestingly, both esters were hydrolyzed to liberate ketorolac free acid upon incubation with intact split-thickness skin. Once again, in a control experiment, no significant chemical hydrolysis of an ester in the 1.15% KCl was noted during the time course of the experiment. This data clearly suggested that the esterases of the split-thickness cadaver skin was responsible for the enzymatic hydrolysis of KDAE and KEE to liberate free acid. A reasonable mass balance of each ester and KA at the end of the 24 h incubation experiment was noted, thus reinforcing the fact that KA was the major metabolic product. The

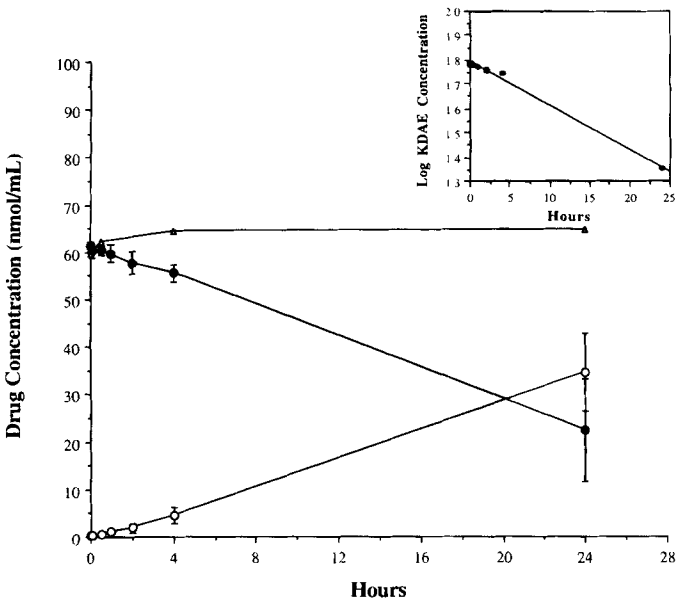

Figure 4—The enzymatic hydrolysis of KDAE upon incubation of human cadaver skin with ester solution at 32 °C. The inset is a log concentration versus time plot showing first-order metabolic degradation kinetics. Key: KEE (closed circle); KA (open circle); control, KDAE in buffer (open triangle).

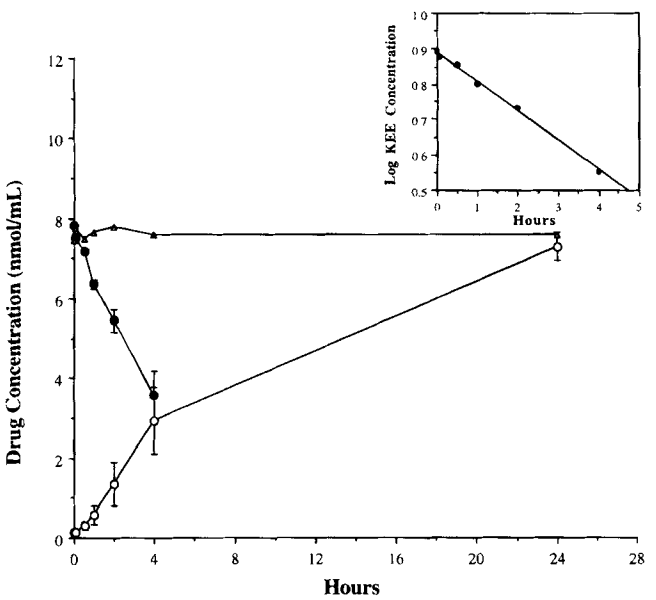

Figure 5—The enzymatic hydrolysis of KEE upon incubation of human cadaver skin with ester solution at 32 °C. The inset is a log concentration versus time plot showing first-order metabolic degradation kinetics. Key: KEE (closed circle); KA (open circle); control, KEE in buffer (open triangle).

localization of the esterases enzyme within various skin layers, however, was not evaluated. It is generally believed that most of the enzyme activity resides in the viable epidermis and dermis layers of the skin.[9]

The log concentration–time plot indicated that the rate of metabolism followed first-order kinetics (see Figures 4 and 5, insets). Table 3 summarizes the rate constant (k) and half-life ($T_{1/2}$) of KADE and KEE. The rate of enzymatic hydrolysis of KDAE upon incubation with split-thickness skin was roughly 6 times longer than that of KEE. This was contrary to serum hydrolysis studies where KDAE hydrolyzed faster than KEE. Such a discrepancy in enzymatic hydrolysis between serum and skin for KDAE and KEE was not clear. It is possible that the form of esterase enzymes that leached out of the intact epidermis and dermis cells (and is responsible

Table 3—Enzymatic Hydrolysis of KDAE and KEE upon Incubation with Split-Thickness Cadaver Skin at 32 °C[a]

Ester	R[b]	k (h^{-1})	$T_{1/2}$ (h)
KDAE	0.99	0.035 ± 0.006	20.2 ± 3.0
KEE	0.99	0.193 ± 0.046	3.7 ± 3.4

[a] Each value is the mean ±SD of three skin donors. Duplicate samples were used for each skin donor. [b] R = correlation coefficient of the linear regression analysis.

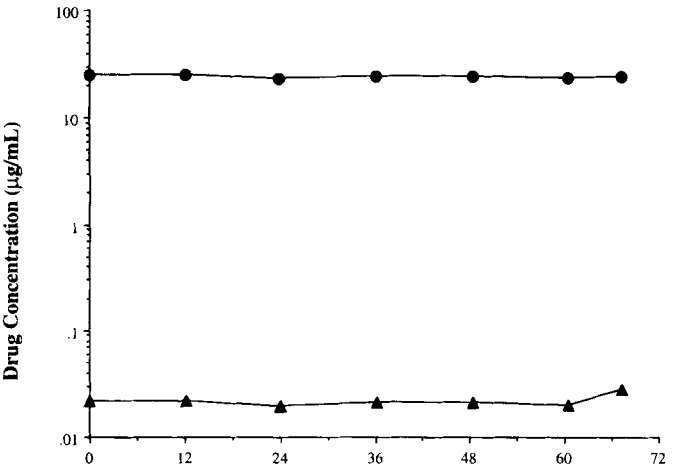

Figure 6—Stability of KDAE (closed circle) and KEE (closed triangle) in 10% PEG-400/water (receiver fluid) at 32 °C.

for the enzymatic hydrolysis of these esters) had a low affinity for KDAE rather than for KEE, which resulted in relatively slower enzymatic hydrolysis of the former ester. In an isolated study, a cadaver skin homogenate (5% in 1.15% KCl isotonic solution) was incubated with an ester solution for about 60 min and the decline in ester concentration as a function of time was monitored in a similar fashion as described for the skin incubation studies. The rate of enzymatic hydrolysis was 1.4×10^{-3} and 1.0×10^{-3} min for KEE and KDAE, respectively (data not shown). Again, the rate of enzymatic hydrolysis by the skin homogenate was slightly higher for KEE than for KDAE. Thus, the substrate specificity for skin esterases and plasma esterases, at least for these two esters, was found to be rather different. Further studies are needed to demonstrate the existence of such a substrate specificity in the enzymatic hydrolysis of these esters.

Skin Permeation of KA and Esters—The skin permeabilities of KA and its two esters though human cadaver skin was evaluated. The 10% PEG-400/water was used as a receiver fluid to maintain a perfect sink condition throughout the skin diffusion experiments.[11,12] Because these esters were susceptible to aqueous hydrolysis, the stability of KDAE and KEE in the receiver fluid was also assessed. Figure 6 shows the kinetics of ester hydrolysis in the receiver fluid incubated for 3 days at 32 °C. The ester concentration in the receptor fluid remained practically unchanged for 3 days. Therefore, 10% PEG-400/water as a receiver fluid was used for the skin permeation experiments.

The skin permeation of KA, KEE, and KDAE from various vehicle compositions was compared. Azone and glyceryl monocaprylate (GMC) as potential skin penetration enhancers were incorporated separately into PG to enhance the skin permeability of these esters. Table 4 summarizes the solubility of KA and esters in various vehicles at 32 °C. No significant chemical hydrolysis of either ester in these organic vehicles was noted at the end of the solubility studies. The

Table 4—Solubilities of Ketorolac Acid and Its Two Esters in Various Vehicles at 32 °C

Vehicle (w/w)[a]	Solubility (mg/mL)[b]		
	KA	KDAE	KEE
PG	53.4	37.1	58.5
PG/GMC (9:1)	69.2	50.5	71.4
PG/Azone (19:1)	66.3	51.9	71.6

[a] PG = propylene glycol; GMC = glyceryl monocaprylate. [b] Each value is the mean of duplicate samples with less than 5% variation.

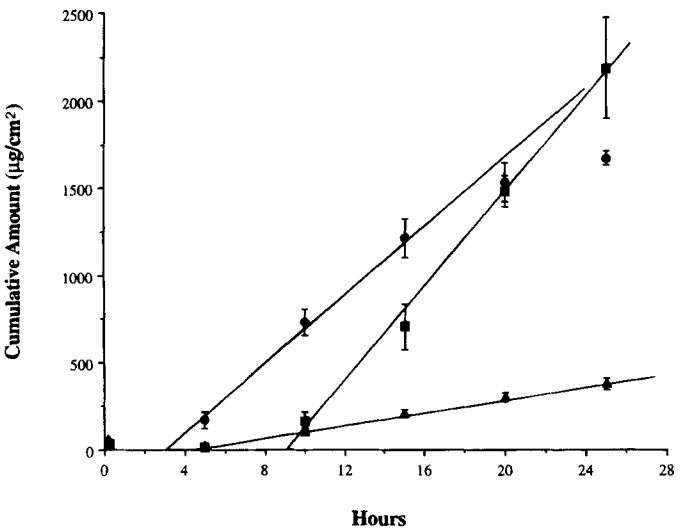

Figure 7—Representative permeation profiles for the diffusion of KA (closed circle), KEE (closed triangle), and KDAE (closed square) though human cadaver skin from PG/GMC (9:1) vehicle mixture at 32 °C.

drug solubility, in general, increased roughly 13% upon addition of either Azone or GMC in PG. The solubility of KEE in all the vehicles tested was higher than those of KA and KDAE. This could be partly because of the lower melting temperature of KEE as opposed to the other two compounds (Table 1).

Figure 7 shows the representative cumulative amount of the drug permeated though cadaver skin as a function of time from a saturated drug solution in the PG/Azone vehicle mixture. The skin data were obtained from a single skin donor to avoid any intersubject variations in the skin permeability. In all cases, after an initial lag time, a steady-state skin flux was attained and maintained for at least 24 h. The mean skin flux was determined from the linear portion of the slope (Table 4). When neat PG was used as a vehicle, no significant differences in the skin fluxes among the three compounds were observed. On the other hand, when Azone and GMC were separately incorporated into the PG, a significant increase in the skin flux was observed. Azone and GMC had a profound effect on the skin flux for KA and KDAE, as opposed to KEE. Indeed, the skin flux of KA and KDAE were enhanced by a factor of 25 and 45 from PG/GMC and PG/Azone vehicle mixtures, respectively, as compared to the neat PG solution. In contrast, only a 6-fold increase in the skin flux of KEE was observed from these vehicle mixtures. The reasons for such a low skin flux of KEE in the presence of Azone and GMC are not clear. It is speculated that the viable epidermal layer of the skin that behaves as an aqueous strata might pose a significant diffusional barrier for a sparingly soluble compound such as KEE.[13] In contrast, the viable epidermis offered very little barrier for the transport of KA and KDAE because these compounds demonstrated relatively higher aqueous solubility at the physiological pH of 7.4.

Table 5—Skin Fluxes of Ketorolac Acid and Its Two Esters through Human Cadaver Skin[a]

	Vehicle Composition (w/w)[b]		
	PG	PG/GMC (9:1)	PG/Azone (19:1)
	J_s (μg/cm²/h)[c]		
KA	2.1 ± 0.3	50.4 ± 10.2	92.2 ± 4.5
KDAE	2.6 (2.9, 2.3)	56.6 ± 6.2	121.0 ± 34
KEE	2.8 ± 0.3	15.1 ± 3.8	18.3 ± 1.4
	T_{lag} (h)		
KA	10.1 ± 2.1	4.0 ± 1.8	2.6 ± 0.5
KDAE	5.1 (4.3, 5.9)	5.4 ± 1.3	9.4 ± 1.3
KEE	4.0 ± 0.9	4.7 ± 1.1	4.0 ± 0.7

[a] All the data were obtained from a single skin donor. Each value is the mean ± SD of three or four diffusion experiments. [b] PG = propylene glycol; GMC = glyceryl monocaprylate. [c] Steady-state skin flux was determined from the linear portion of the cumulative amount–time plot.

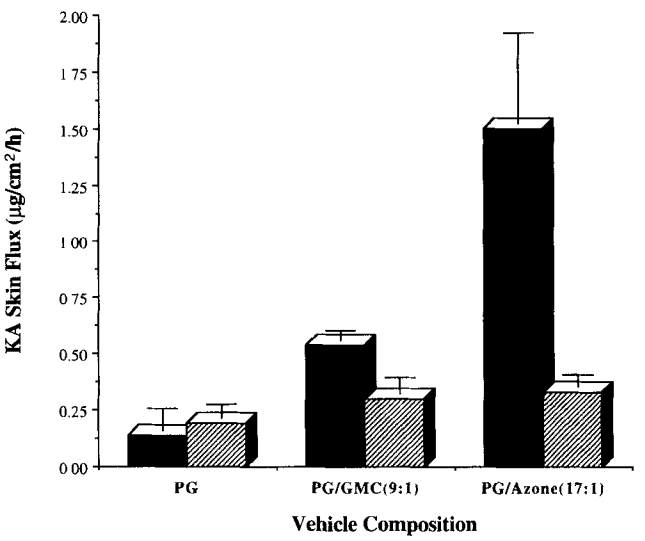

Figure 8—The skin flux of KA following application of KDAE (closed bar) and KEE (hatched bar) transdermal solution in the donor compartment. PG = propylene glycol; GMC = glyceryl monocapyrlate.

The lag times of KA, KEE, and KDAE from the three transdermal solutions are also presented in Table 5. The lag time of KA reduced from 10 h in PG to 2.6 h in PG/Azone, while the lag time of KEE remained fairly constant in all the solutions tested. In contrast, the lag time of KDAE in the PG/Azone solution increased by a factor of 2 as compared to the neat PG and PG/GMC solutions. Further investigation is needed to explain such a discrepancy in lag time between the free acid and the esters.

The concurrent skin flux of KA following the application of each ester solution was also monitored. In all cases, KA was detected in the receiver fluid. Figure 8 shows the mean steady-state skin flux of KA following application of various ester solutions. As the skin flux of KDAE increased (Table 5), the concurrent skin flux of KA also increased. Except for the PG formulation, the skin flux of KA from KDAE solutions was significantly higher than that from the KEE solution. The extent of ester hydrolysis by skin enzymes in vivo, however, is anticipated to be higher than that of the heat-separated epidermis used in the in vitro skin permeation studies. Nonetheless, once the ester enters the systemic circulation, the ester prodrug will eventually be metabolized by serum esterases to liberate the active drug, KA.

Conclusions

In summary, we demonstrated in vitro serum hydrolysis of ketorolac esters. KA was the major metabolite of such enzymatic hydrolysis. The serum half-life of KDAE was about 3 times shorter than KEE. Upon incubation of human cadaver skin with the ester solution, the enzymatic hydrolysis of ester to free acid was observed. The enzymatic hydrolysis rates of the skin was several times lower than that of serum. The in vitro skin flux of KA and its two esters from a PG solution was virtually the same. On the other hand, the skin flux of KA and KDAE from PG/GMC and PG/Azone solutions was several times higher than that of KEE. Biotransformation of KEE and KDAE to KA during the in vitro diffusion though human epidermis was very low, suggesting that a large fraction of intact ester could be transported into the cutaneous microcirculation and could eventually be converted to the parent drug by the serum esterases.

References and Notes

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