Absorption of Transdermally Delivered Ketorolac Acid in Humans

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Received June 2, 1994, from the *Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304.* Accepted for publication publication September 29, 1994[⊗].

Abstract □ Transdermal delivery of ketorolac acid, a potent analgesic, through human skin in vitro and in vivo was evaluated. The following three transdermal solutions were selected to study the in vitro skin permeation rate of ketorolac acid: formulation A, isopropyl alcohol:water: isopropyl myristate (IPA/water/IPM; 11:7:1); formulation B, ethanol: propylene glycol:isopropyl myristate (ET/PG/IPM; 11:7:2); and formulation C, IPM/capmul (glyceryl mono- and dicaprylate; Monoctanoin). The permeation of ketorolac acid through cadaver skin from a saturated drug solution was evaluated at 32 °C with a modified Franz diffusion cell. The in vitro skin fluxes were 180, 177, and 14 μg/cm^2/h for fomulations A, B, and C, respectively. The systemic bioavailability of ketorolac acid from three transdermal formulations was evaluated in nine healthy subjects in a randomized three-way crossover fashion. Hill Top chambers were used as prototype dermal delivery devices to load the drug solution. This procedure was followed by the immediate application of devices to human subjects for 24 h. Blood samples were collected at various time intervals up to 48 h, and the samples were assayed by HPLC. The basic pharmacokinetic parameters were derived from the drug plasma concentration versus time plot. The maximum drug plasma concentrations were 1.265, 0.696, and 0.092 μg/mL for formulations A, B, and C, respectively. Formulation A provided the highest in vitro and in vivo transdermal delivery rate among the three formulations studied. An excellent correlation between the in vitro steady-state skin flux and the area under the curve of in vivo plasma drug concentration versus time was observed for all the three formulations.

Ketorolac tromethamine is a nonsteroidal agent with potent analgesic and moderate anti-inflammatory activities. Clinical studies with ketorolac tromethamine indicated a single-dose efficacy greater than that of morphine, meperidine, and pentazocine in moderate to severe post-operative pain, with some evidence of a more favorable adverse-effect profile than morphine or meperidine.[1] In addition, unlike narcotic analgesics, ketorolac does not pose potential addiction problems and respiratory depression that are commonly associated with narcotic analgesics therapy; therefore, ketorolac is a relatively more favorable therapeutic agent for the management of post-operative pain.

Ketorolac (as the tromethamine salt) is currently administered intramuscularly (im), intravenously, orally, or as a topical ophthalmic solution. Because the biological half-life of ketorolac acid was reported to be 4–6 h, frequent dosing was necessary to sustain the action of the drug to alleviate pain in post-operative patients.[1] Transdermal delivery of ketorolac appears to be an attractive mode of administration for systemic action of the drug, and for an extended period of time. Indeed, Yu et al.[2] demonstrated the percutaneous absorption and systemic bioavailability of ketorolac acid and ketorolac tromethamine salt from various vehicle systems in the rhesus monkey. In that report, various transdermal formulations containing at least one potential skin penetration enhancer dissolved in propylene glycol were loaded into a Hill Top chamber dermal delivery system that was immediately applied to rhesus monkeys and held in place for ~24 h. The feasibility of transdermal delivery of ketorolac in the rhesus monkey was demonstrated by monitoring the drug plasma levels over 48 h. Recently, we reported the skin permeability of ketorolac acid and its two esters through human cadaver skin in a study designed to improve the skin permeation of ketorolac acid through a prodrug approach.[3] The prodrug approach utilized two esters but was not very successful because the skin fluxes of these esters were either low or very similar to that of the parent drug.[3] Therefore, in this study, we evaluated various potential skin penetration enhancers to improve systemic bioavailability of ketorolac acid in humans.

The principal objectives of this report are to evaluate the in vitro permeability of ketorolac acid through human cadaver skin from three promising vehicle formulations, to evaluate the pharmacokinetics of ketorolac acid in healthy humans following application of a transdermal solution, and to assess in vitro/in vivo correlations.

Experimental Section

Material—Ketorolac acid was obtained from the Institute of Organic Chemistry, Syntex (Palo Alto, CA). Isopropyl alcohol (IPA), isopropyl myristate (IPM), ethanol (95%, ET), and propylene glycol (PG) were purchased from Sigma Chemical (St. Louis, MO) and Aldrich Chemical (Milwaukee, WI). Capmul (glyceryl mono- and dicaprylate; monoctanoin) was purchased from Capitol City Products (Columbus, OH). Hill Top chamber delivery devices were purchased from The Hill Top Companies (Cincinnati, OH). All other chemicals used in the study were of analytical reagent grade and used as such without any further purification.

Preparation of Drug Solution and Solubility Determination—The vehicle compositions are summarized in Table 1. Although both polar (i.e., water, IPA, and ethanol) and nonpolar (i.e., IPM) vehicles were mixed at different proportions, the final drug solution was a clear homogeneous mixture to avoid any complication that might arise while interpreting the permeation data. In all cases, a saturated drug solution with little excess of solute was used to ensure unit thermodynamic activity throughout the diffusion experiments. The solubility of ketorolac acid in each vehicle mixture at 32 °C was determined by an HPLC assay.

In Vitro Skin Permeation Studies—Human cadaver skin was used for the permeation studies. Samples of split-thickness skin were removed from the abdomen of human cadavers within 48 h postmortem with a dermatome set at 300 μm. The skin was cut into circular patches and checked immediately for any leaks prior to the application of the drug solution. The skin was then mounted carefully on a modified Franz diffusion cell and fastened with a rigid clamp. The active diffusion area of the cells was 2.01 cm^2. The receiver compartments were filled with an isotonic phosphate buffer (pH 7.4, 22 mL) containing 0.002% gentamicin as an antibacterial agent. The receiver fluid was stirred throughout the experiment to ensure a suitable mixing of solutions. The skin samples were allowed to equilibrate with the buffer solution for ~30 min before charging the donor solution. The blank receiver samples were subjected to HPLC assay to ensure the absence of any interfering peaks that might have been leached out from the skin into the receiver fluid. The donor compartment was then charged with 2 mL of saturated ketorolac acid solution and covered immediately with a plastic film (Sara Wrap) to prevent any significant loss of volatile components from the transdermal solution. At predetermined time intervals, 1 mL of receiver solution was withdrawn and replaced with an equal amount of fresh buffer solution. The duration of the skin diffusion experiments was 28 h. Ketorolac acid in the receiver fluid sample was assayed by HPLC.

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Table 1—Composition of Ketorolac Acid Transdermal Formulations

Formulation Identification	Vehicle Composition (w/w)	Ketorolac Acid, mg/mL[a]
A	IPA/water/IPM (11:7:1)[b]	69.5
B	ET/PG/IPM (11:7:2)	95.5
C	IPM/Capmul (4:1)	8.2

[a] In all cases, a saturated drug solution with an excess of solute was used to maintain a unit thermodynamic activity throughout skin diffusion experiments. [b] pH = 4.5.

Study Design in Healthy Humans—Three transdermal ketorolac acid solutions (Table 1) were evaluated in nine healthy male and female subjects to determine the systemic bioavailability of each transdermal ketorolac solution. For every subject, 2.5 mL of the ketorolac solution was loaded uniformly onto a nonwoven pad of the Hill Top chamber that had an active diffusion area of 20 cm^2 and was attached to an overlay adhesive tape. The Hill Top chamber was weighed and applied immediately to the back of each subject; Hill Top chambers were held in place on the skin for 20 s and then left on occluded for 24 h. Following a washout period of ~1 week, all the subjects received the next formulation.

Blood samples (7 mL each) were collected in heparinized tubes immediately prior to application of the Hill Top chambers and at the following times thereafter: 0.5, 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, and 48 h. The plasma was separated from the whole blood by centrifugation at 3000 rpm for 10 min, and the plasma was transferred into polypropylene tubes. The plasma was stored at −20 °C for later HPLC assay.

The Hill Top chambers were removed after the 24-h application and assayed later for ketorolac acid content. The drug was extracted from the Hill Top chamber simply by placing the chamber in a glass jar containing 50 mL of HPLC mobile phase (see *Assay Procedure*). The solution was sonicated for 2 min to completely release the drug from the Hill Top chamber into the mobile phase. A 1-mL sample was withdrawn and transferred into a small vial, and the sample was assayed by HPLC.

Assay Procedure—The ketorolac sample was assayed by HPLC (SP 8800 precision isocratic pump, Spectra Physics) with UV detection at 314 nm. A Spherisorb ODS I column (5 μm; 25 cm × 4.5 mm, Alltech) and acetonitrile:water:acetic acid (44:58:1; pH 3.0) as a mobile phase were used for chromatographic resolution. The flow rate was 1 mL/min, and the peak height was integrated by a Chrom-Jet Integrator (Spectra Physics). Various concentrations of ketorolac tromethamine in methanol were used to construct a calibration curve. Calibration curves were obtained by plotting the peak height of the authentic sample as a function of ketorolac free acid concentrations. Ketorolac acid concentrations in plasma samples were determined by a similar HPLC procedure.[4]

Data Analysis—*In Vitro*—The in vitro skin flux was determined as follows[3]:

$$J_s = 1/A(dM/dt) \quad (1)$$

where J_s is the skin flux (μg/cm^2/h), A is the diffusion area (cm^2), and dM/dt is the amount of drug permeated per unit time. The in vitro lag time was determined by extrapolating the linear portion of the cumulative amount versus time plot to the abscissa.

In Vivo—The following pharmacokinetic parameters (in humans) were computed for each formulation: time to maximum concentration (T_{max}); peak plasma concentration (C_{max}); plasma half-life ($t_{1/2}$); and the area under the curve of concentration versus time from 0 to 24 h (AUC_{0-24}) and from 0 to infinity ($AUC_{0-\infty}$) were determined with the linear trapezoidal rule. Statistical analysis was performed with analysis of variances (ANOVA).

Results and Discussion

In Vitro Skin Flux of Ketorolac Acid—Typical cumulative amount–time profiles of ketorolac acid through human cadaver skin from the three ketorolac transdermal formulations are shown in Figure 1. It is interesting to note that the time to reach steady-state skin flux of ketorolac acid from formulation A (IPA/water/IPM) was remarkably shorter than

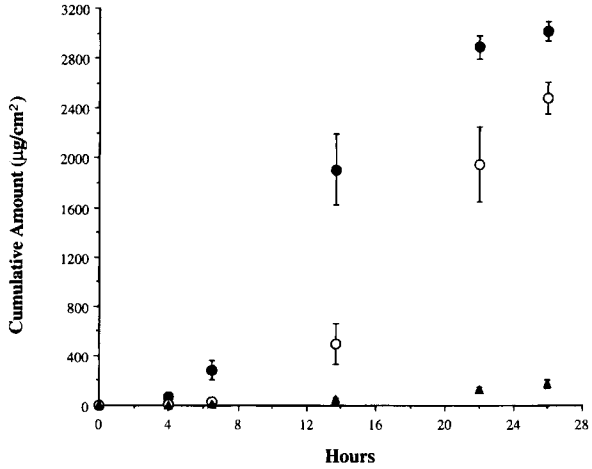

Figure 1—Representative in vitro permeation profiles of ketorolac through cadaver skin from three transdermal vehicle formulations at 32 °C. Key: (●) formulation A; (○) formulation B; and (▲) formulation C. Each data point is the mean ±SD of three to four diffusion experiments.

Table 2—In Vitro Skin Permeation of Ketorolac Acid from Three Transdermal Formulations through Human Cadaver Skin at 32 °C[a]

		J_s, μg/cm^2/h			T_{lag}, h		
Skin Donor	N[b]	A	B	C	A	B	C
1	4	165 ± 8	145 ± 9	8.7 ± 1.7	3.7 ± 0.6	7.5 ± 0.4	6.8 ± 0.2
2	3	198 ± 9	198 ± 8	14.4 ± 1.4	3.0 ± 0.5	4.4 ± 0.7	6.1 ± 0.2
3	3	177 ± 2	187 ± 1	18.9 ± 2.5	0.6 ± 0.5	2.9 ± 0.5	5.7 ± 0.3
Mean		180	177	14.0	2.4	5.1	6.2
SD		17	28	5	1.6	2.6	0.6

[a] Transdermal solution compositions are shown in Table 1. [b] Number of diffusion experiments.

that from the other two vehicle formulations studied. In all cases, however, a steady-state skin flux was maintained for at least 24 h and no significant depletion of the drug from the donor compartment was observed at the end of the diffusion study (28 h).

The skin flux and lag time of ketorolac acid from the three transdermal vehicle formulations are summarized in Table 2. Three different skin donors were used to demonstrate intraindividual variations in skin flux. The mean skin fluxes of ketorolac acid were 180, 177, and 14 μg/cm^2/h for formulations A, B, and C, respectively. These skin fluxes were several-folds higher than that from pure water (skin flux typically ranged from 1 to 2 μg/cm^2/h, data not shown), suggesting that these vehicles act as skin penetration enhancers. Thus, hydrooxylated nonaqueous vehicles, such as ET, IPA, and PG, in combination had the ability to enhance the skin permeation of ketorolac acid through human cadaver skin. Although the drug solubility in ET/PG/IPM vehicle (95.5 mg/mL) was higher than that in IPA/water/IPM vehicle (69.5 mg/mL), the in vitro skin flux was virtually the same for these two formulations. The skin flux of ketorolac acid from formulation C, however, was several times lower than that from formulations A and B. This result implies that a hydrooxylated nonaqueous vehicle was an essential component of the transdermal formulation to achieve a maximal skin flux of ketorolac acid. Although the mechanism of skin penetration enhancement by a hydrooxylated nonaqueous vehicle is not quite clear, it is speculated that fluidization of the lipids of the stratum corneum (which is generally believed to be the principle diffusion barrier for a majority of drugs) by the hydrooxylated vehicle might have contributed to an enhanced transport of the ketorolac acid through the skin. Although formulations A and B provided virtually the same

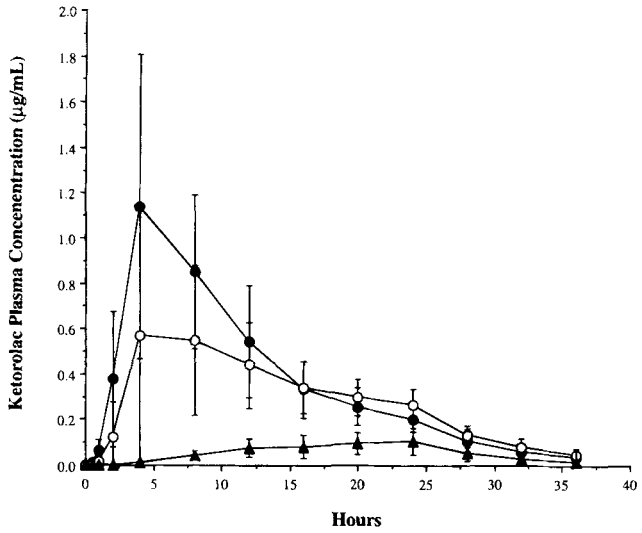

Figure 2—Ketorolac plasma concentration following application of Hill Top chamber for 24 h in nine healthy subject. The patches were removed after 24 h of application. Key: (●) formulation A; (○) formulation B; and (▲) formulation C. Each plasma data point is the mean of nine healthy humans.

skin flux in vitro, the T_{lag} was twice as fast for formulation A as for formulation B.

Plasma Profiles of Ketorolac in Humans—Profiles of plasma concentration of ketorolac versus time following the application of a Hill Top chamber loaded with 2.5 mL of the saturated drug solution are shown in Figure 2. The plasma levels of ketorolac acid increased steadily, and the C_{max} was attained within 4–6 h following the applications of formulations A and B. In the case of formulation A, however, a rapid decline in the plasma drug concentration after reaching a C_{max} at ~5 h was observed. Such a decline in ketorolac plasma concentration was most likely because of a relative fast depletion of IPA from the IPA/water/IPM ternary vehicle mixture. Although the ET/PG/IPM vehicle mixture exhibited somewhat similar trends in the plasma concentration–time profile, a relatively shallow decline in the drug plasma levels after the attainment of C_{max} was observed. This result implied that the depletion of PG and ET from the ET/PG/IPM vehicle was relatively slower than that of IPA from the IPA/water/IPM vehicle mixture. This is not surprising, because the permeability of IPA through cadaver skin was reported to be roughly two times greater than that of ET and PG.[5]

An interesting trend in the ketorolac plasma profiles following application of the drug solution to subjects was observed. For all nine subjects receiving formulation A, ketorolac acid in the plasma samples was detected within 1 h of application of the Hill Top chamber. This was not surprising, because formulation A provided the shortest *in vitro* steady-state lag time among the ketorolac transdermal formulations studied. In contrast, formulation C did not show any detectable ketorolac in the plasma samples until 8 h, which is in close agreement with in vitro findings that the mean steady-state lag time of ketorolac was ~6.2 h (Table 2). The decline in ketorolac blood levels upon removal of Hill Top chamber after 24 h, however, was virtually the same for all three vehicle formulations.

The mean pharmacokinetic parameters of ketorolac acid are summarized in Table 3. The mean terminal half-lives of ketorolac, as estimated from the terminal portion of the semilog of plasma concentration–time plot, ranged from 4.6 to 5.9 h for all three transdermal formulations. These results are virtually the same as those reported for ketorolac following a bolus im injection.[1] Our results clearly suggest that ketorolac acid had very little or no skin depot in the stratum

Table 3—Mean Pharmacokinetic Parameters of Ketorolac Acid Following Application of Hill Top Chamber Loaded with Transdermal Solutions in Nine Healthy Subjects for 24 h[a]

Pharmacokinetic Parameter	Transdermal Formulation A	B	C	p Value
T_{max}, h	5.3 (38)	7.1 (47)	22.2 (10)	0.0001
C_{max}, μg/mL	1.27 (45)	0.70 (72)	0.09 (63)	0.0001
$t_{1/2}$, h	5.9 (30)	5.8 (24)	4.6 (35)	0.0852
AUC_{0-24}, μg·h/mL	12.34 (34)	8.94 (51)	1.24 (65)	0.0001
$AUC_{0-\infty}$, μg·h/mL	13.89 (33)	10.90 (46)	2.02 (49)	0.0001

[a] Each value is the mean (± %CV) of nine subjects.

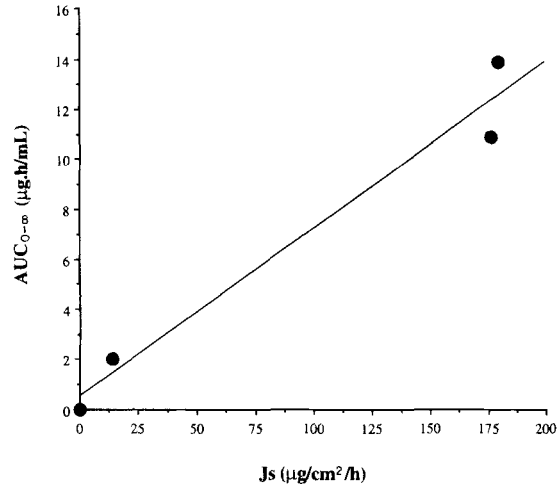

Figure 3—Relationship between in vitro skin flux (J_s) and in vivo plasma $AUC_{0-\infty}$ in healthy humans for three transdermal formulations with a correlation coefficient of 0.983.

corneum upon removal of the Hill Top chamber because of its relatively high dermal clearance in vivo. The T_{max} was significantly shorter for formulations A and B (<7 h) than for formulation C (22 h, $p = 0.0001$). The C_{max} values were 1.265, 0.696, and 0.092 μg/mL for formulations A, B, and C, respectively. The AUC was determined for each formulation with the linear trapezoidal rule. The AUC_{0-24} for formulations A and B, respectively, were 6.9 and 5.4 times higher than that for formulation C ($p = 0.0001$).

The relationship between in vitro skin flux (mean of three skin donors) and in vivo $AUC_{0-\infty}$ in nine healthy humans is depicted in Figure 3. An excellent correlation ($r = 0.98$) between J_s and $AUC_{0-\infty}$ was observed. These results clearly suggest that human cadaver skin is a good skin model to predict plasma levels of ketorolac in humans from the three vehicle formulations studied.

The residual amount of ketorolac acid in the used Hill Top chamber is summarized in Table 4. The initial drug amount in the Hill Top chamber was determined from the drug concentration multiplied by the volume of the solution loaded in each patch. The initial loading amount minus the residual amount in the used Hill Top chamber provided the approximate amount of ketorolac depleted or delivered (Δ, in mg) from the Hill Top chamber to the skin. The amount of drug depleted from the patch could also be assumed to be the amount of ketorolac delivered into the systemic circulation, provided there was no significant leakage of drug solution and negligible skin metabolism during the diffusion process. The mean amounts of ketorolac depleted (or delivered) from the Hill Top chamber were 30.0, 34.0, and 7.9 mg for formulations A, B, and C, respectively. It is rather interesting to note that even though the amount of the drug depleted from formulation B was ~13% higher than that from formulation A, the mean

Table 4—Residual Amount of Ketorolac Acid in the Used Hill Top Chamber

	Formulation								
	A			B			C		
Subject Number	Initial	Final	Δ[a]	Initial	Final	Δ	Initial	Final	Δ
1	150.5	118.3	32.2	178.5	149.3	29.2	17.6	11.7	5.9
2	123.1	93.6	29.5	186.1	131.1	55.0	17.9	7.0	10.9
3	129.9	94.9	35.0	186.4	159.6	26.9	16.7	10.1	6.6
4	129.6	104.0	25.6	185.2	124.1	61.1	18.2	8.8	9.4
5	129.5	112.1	17.4	184.3	166.0	18.3	18.9	12.4	6.5
6	134.8	93.9	40.9	181.8	151.8	30.0	16.4	8.0	8.4
7	123.5	99.3	24.2	188.3	150.4	37.9	18.5	10.6	7.9
8	134.2	88.3	45.9	186.9	154.7	32.2	17.0	9.1	7.9
9	125.7	106.8	18.9	176.3	161.3	15.0	17.2	9.9	7.2
Mean	131.2	101.3	30.0	183.8	149.8	34.0	17.6	9.7	7.9
SD	8.3	9.8	9.6	4.1	13.8	15.4	0.8	1.7	1.6

[a] Amount depleted or delivered from each transdermal patch (initial – final).

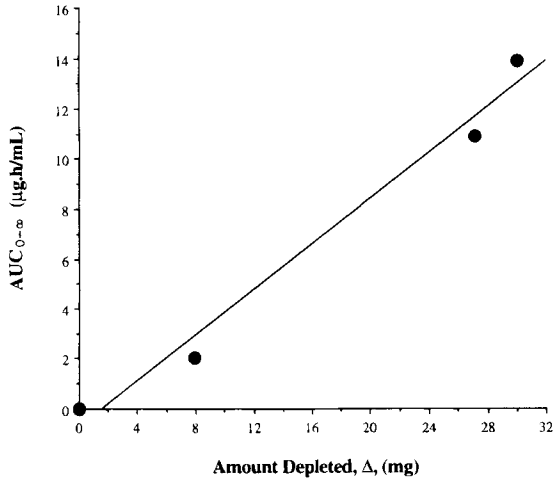

Figure 4—Relationship between amount of drug depleted (△) and in vivo plasma $AUC_{0-\infty}$ in healthy humans for three transdermal formulations with a correlation coefficient of 0.989.

$AUC_{0-\infty}$ of the former formulation was only 70% of the latter formulation. Review of the residual patch depletion data for formulation B indicates that two subjects (subject 2 and 4) had unexpectedly high depletion values (Table 4). Such a high depletion for the two subjects was most likely due to leakage of the transdermal solution from the Hill Top chamber during the 24-h wearing period. The mean $AUC_{0-\infty}$ of the remaining seven subjects for formulation B was 27.1 mg, which is ~11% lower than that of formulation A. The high depletion for two subjects explains the low mean $AUC_{0-\infty}$ value for formulation B. Furthermore, the intersubject variability in the percutaneous absorption of ketorolac acid ranged from 32 to 45% (this may be partly because of inherent variability in the permeability of human skin to ketorolac acid).

The relationship between the amount of ketorolac acid depleted or delivered from the Hill Top chamber and plasma ($AUC_{0-\infty}$ is shown in Figure 4 (noted that a corrected depletion value for formulation B as just mentioned, was used for this correlation plot). An excellent correlation (r = 0.98) between amount delivered and $AUC_{0-\infty}$ was observed for three transdermal formulations studied. These results clearly suggest that one could predict plasma AUC of ketorolac acid from the patch depletion data provided there is no drug solution leakage during the entire period of wearing the chamber.

Conclusions

We have demonstrated the transdermal bioavailability of ketorolac acid in healthy subjects following application of three different transdermal formulations. The in vitro skin fluxes of ketorolac from the three vehicle formulations were in the following order: formulation A (IPA/water/IPM) > formulation B (ET/PG/IPM) > formulation C (IPM/Capmul). Although the in vitro skin fluxes of ketorolac acid were virtually the same for transdermal formulations A and B, the former formulation provided the shortest lag time among the three formulations studied. Formulation A provided the highest plasma C_{max} and $AUC_{0-\infty}$ among the three transdermal formulations studied. The terminal half-life of ketorolac acid was virtually the same for the two transdermal formulations, and no obvious skin depot effect of the drug was observed. An excellent correlation between the in vitro skin flux and in vivo $AUC_{0-\infty}$ was observed, reinforcing the fact that cadaver skin can serve as a good skin model for screening ketorolac transdermal formulations.

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