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Report

Percutaneous Absorption of Nicardipine and Ketorolac in Rhesus Monkeys

Diana Yu,[1,3] Lynda M. Sanders,[1] George W. R. Davidson III,[1] Martha J. Marvin,[1] and Teck Ling[2]

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Vehicle effects on the percutaneous absorption of nicardipine base, nicardipine hydrochloride, ketorolac acid, and ketorolac tromethamine were determined using the rhesus monkey as an *in vivo* model for human skin penetration. Vehicles investigated included blends of propylene glycol, trimethylene glycol, ethanol, Azone, Tween 20, water, and long-chain fatty acids. Formulations were prepared such that the compound dose, application area, and percentage saturation of the compound in the vehicle were held constant. Variations in absorption of the compounds were therefore attributable to vehicle effects. Each formulation was applied to three monkeys for a period of 24 hr using 10 Hill Top Chambers. Plasma samples were taken at appropriate intervals for 36 to 48 hr. The results indicated that trimethylene glycol and Tween 20 did not enhance absorption of the test compounds despite claims by other investigators. Azone and ethanol provided moderate enhancement of both the rate and the extent of absorption, while long-chain fatty acids in combination with propylene glycol significantly enhanced penetration. In general, higher fluxes were observed with the more lipophilic compounds nicardipine base and ketorolac acid as compared to the hydrochloride and tromethamine salts.

KEY WORDS: percutaneous absorption; nicardipine; ketorolac; penetration enhancers; vehicle effects.

INTRODUCTION

An analogy has often been made between the percutaneous absorption of drug compounds and solute diffusion through a membrane (1–6). The complexities of human skin are generally ignored and the stratum corneum is considered to be the rate-limiting membrane through which the solute must penetrate. Based on irreversible thermodynamics, the steady-state flux, or diffusion rate per unit area, of a solute through a membrane may be generally expressed as

$$J = -L\frac{d}{dx}\left(\frac{u}{T}\right) = -L\frac{RT}{a}\frac{d}{dx}\left(\frac{a}{T}\right) \qquad (1)$$

where J is the flux, L is the Onsager phenomenological coefficient for diffusion, u is the chemical potential of the solute, T is the temperature, a is the thermodynamic activity of the solute, and R is the ideal gas constant (7,8). At a constant temperature, therefore, the driving force behind the diffusional process is the thermodynamic activity gradient across the membrane, da/dx. Equation (1) simplifies to the well-known Fick's first law of diffusion only when it is also assumed that all solutions are ideal:

$$J = \frac{LR}{a}\frac{da}{dx} = -D\frac{dc}{dx} \qquad (2)$$

where c it the concentration of the solute and D is the diffusion coefficient of the solute. This assumption of ideality has often been ignored in discussions on membrane permeability, resulting in undue importance being placed on the concentration gradient.

The concept of activity gradient-driven diffusion becomes especially important when considering vehicle effects on percutaneous absorption. Since the thermodynamic activity of a solute is maximized at the solubility limit, the activity gradient and, therefore, the diffusional flux may be maximized by using a saturated donor phase. Absorption enhancement due to vehicle effects, therefore, becomes apparent when nonidentical fluxes are obtained with different vehicles at a constant thermodynamic activity (not a constant concentration) (9).

In this study, vehicle effects on the percutaneous absorption of drug compounds were determined using the rhesus monkey as a model for human skin penetration. Studies have indicated that the rhesus monkey is a good animal model for this purpose (10). Nicardipine (free base), nicardipine hydrochloride, ketorolac (free acid), and ketorolac tromethamine were selected as test drug compounds (see Fig. 1).

Several vehicle solvents were selected based on poten-

[1] Institute of Pharmaceutical Sciences, Syntex Research, 3401 Hillview Avenue, Palo Alto, California 94304.

[2] Bioanalytical and Metabolic Research, Syntex Research, Palo Alto, California 94304.

[3] To whom correspondence should be addressed.

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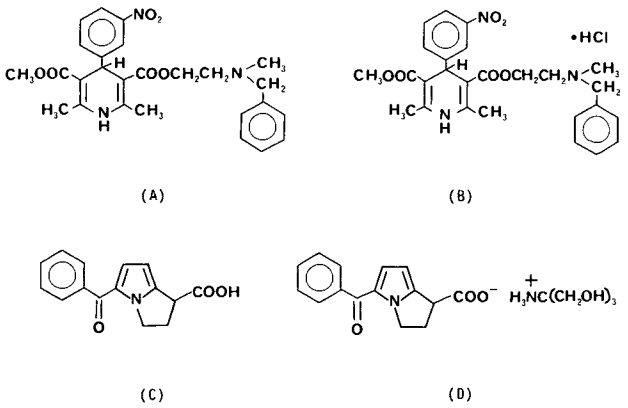

Fig. 1. Drug compounds used in this study: (A) nicardipine; (B) nicardipine hydrochloride; (C) ketorolac; (D) ketorolac tromethamine.

tial absorption enhancement. In particular, propylene glycol has been reported to enhance percutaneous absorption in some cases (11). Trimethylene glycol has been claimed as a skin penetration enhancer for nifedipine and nicardipine (12). Ethanol has been shown to increase the skin permeation rate of estradiol (13). Cooper has published results indicating that blends of polar solvents, such as propylene glycol, and long-chain fatty acids, such as linoleic and oleic acids, increase the permeability of lipophilic compounds (14). Azone (1-dodecylazacycloheptan-2-one) is claimed to be a penetration enhancer and several investigators have reported skin penetration enhancement in the presence of surfactants (11,15–17). Vehicles composed of a combination of two or three of these solvents were chosen for use in this study.

MATERIALS AND METHODS

Materials. Nicardipine, nicardipine hydrochloride, ketorolac, and ketorolac tromethamine were provided by Syntex Corporation (Palo Alto, Calif.). Propylene glycol, USP (PG) (Syntex Corporation, Palo Alto, Calif.), trimethylene glycol (TMG) (1,3-propanediol; Eastman Kodak Co., Rochester, N.Y.), linoleic acid (LA) (Sigma Chemical Co., St. Louis, Mo.), oleic acid, NF-FCC (OA) (J. T. Baker Chemical Co., Phillipsburg, N.J.), Azone (AZ) (Nelson Research and Development, Irvine Calif.), Tween 20 (TW) (ICI Americas, Inc., Wilmington, Del.), and alcohol, USP (95% ethanol) (E) (190-proof alcohol, USP; U.S. Industrial Chemicals), were used as obtained. Twice deionized water (W) was used in some vehicles. Hill Top Chambers (HTCs) (18) were used as received from Hill Top Research.

Solubility Studies. Although the skin temperature of rhesus monkeys is higher, solubility studies were carried out at 25°C. In assuming maximum thermodynamic activity, therefore, the temperature coefficient of solubility for each compound was also assumed to be identical in each of the vehicles. The solubilities of nicardipine and nicardipine hydrochloride in PG/W, TMG/W, PG/LA, PG/W/AZ, PG/LA/W, and E/W were determined in triplicate by placing excess compound with each vehicle blend in a screw-capped vial. The vials were then rotated for 3 days in a 25°C water bath. Upon removal, the suspensions were filtered through 0.45-μm membrane filters, diluted in mobile phase, and assayed for drug content by high-performance liquid chromatography (HPLC). The solubilities of ketorolac and ketorolac tromethamine in PG/W, PG/LA, PG/OA, PG/W/TW, and E/W were also determined.

Percutaneous Absorption Studies. Formulations were chosen based on the results of the solubility studies. A 10-ml solution of each formulation was prepared, the appropriate volume of which was then loaded into each HTC using a 1000-μl glass syringe fitted with an 18-gauge needle.

For each formulation, three female rhesus monkeys (*Macaca mulatta*) weighing 6 to 9 kg were used. Chest hair was clipped closely rather than shaved in order to ensure that the stratum corneum remained intact. Prior to dosing, the animals were lightly anesthetized with 5 to 8 mg/kg ketamine. Each formulation was then applied over a total chest area of 27 cm^2 using 10 HTCs per monkey held in place by a single strip of adhesive tape. Three-milliliter blood samples were taken from the saphenous vein prior to dosing and at appropriate intervals over a period of 36 to 48 hr. The monkeys were restrained in metabolism chairs during the 24 hr application period. Following HTC removal, the application site was washed with soap and water. Plasma levels of compound were determined by HPLC (ketorolac and ketorolac tromethamine) (19) or a capillary column gas chromatographic (GC) method with electron capture detection (nicardipine and nicardipine hydrochloride) (20).

Compound Remaining Assay. Following removal of the HTCs from each monkey, the cotton swatches from 5 of the 10 HTCs were removed, placed in a 50-ml beaker with 30 ml of acetonitrile, methanol, or alcohol, U.S.P., and sonicated for 15 min. The extract was then filtered through a 0.45-μm membrane filter into a 100-ml volumetric flask. After two additional rinses, the extract solution was made to volume, diluted in mobile phase, and assayed for drug content by HPLC.

HPLC Analytical Methods. For nicardipine and nicardipine hydrochloride, the retention time was 7.8 min using 50:50 CH_3CN:0.05 *M* KH_2PO_4 at 1.0 ml/min through a Whatman Partisil 5 ODS-3 column. Detection was by UV absorption at 237 nm. For ketorolac and ketorolac tromethamine, the retention time was 6.4 min using 40:60:0.2 CH_3CH:H_2O:CH_3 COOH at 1.3 ml/min through an ASI-C8 (10 μm) column. Detection was by UV absorption at 254 nm.

RESULTS AND DISCUSSION

Solubility Studies. Since the development of therapeutically effective formulations was the ultimate goal, an appropriate compound dose, *d*, was selected for each compound. Both the area of application and the allowable donor phase volume range, *v*, were fixed based on the use of 10 HTCs for formulation application. Therefore, in order to maintain a constant percentage saturation, *f*, in the vehicle, a solubility limit, *s*, falling within a specified range of values was required:

$$0.286\,\frac{d}{f} \leq \left(s = \frac{d}{vf}\right) \leq 2.00\,\frac{d}{f} \qquad (3)$$

Table I. Doses and Target Solubility Ranges

Compound	Dose (mg)	Desired % saturation	Solubility range (mg/ml)[a]	Preferred solubility (mg/ml)[b]
Nicardipine	93	90	29.5–207	34.4
Nicardipine hydrochloride	100	90	31.8–222	37.0
Ketorolac	60	95	18.0–126	21.0
Ketorolac tromethamine	85	95	25.6–179	29.8

[a] Based on Eq. (3).
[b] Based on Eq. (3); v = 3.0 ml is optimal based on HTC design.

where v = 0.5 to 3.5 ml. For example, for nicardipine base, the desired dose, d, was 93 mg. With v = 0.5 to 3.5 ml and f = 0.90, solubilities ranging from 29.5 to 207 mg/ml were required. Doses and target solubility ranges for these compounds are shown in Table I. Solutions rather than suspensions were used in order to avoid the possibility of solid drug dissolution being the rate-limiting step in absorption of the compounds.

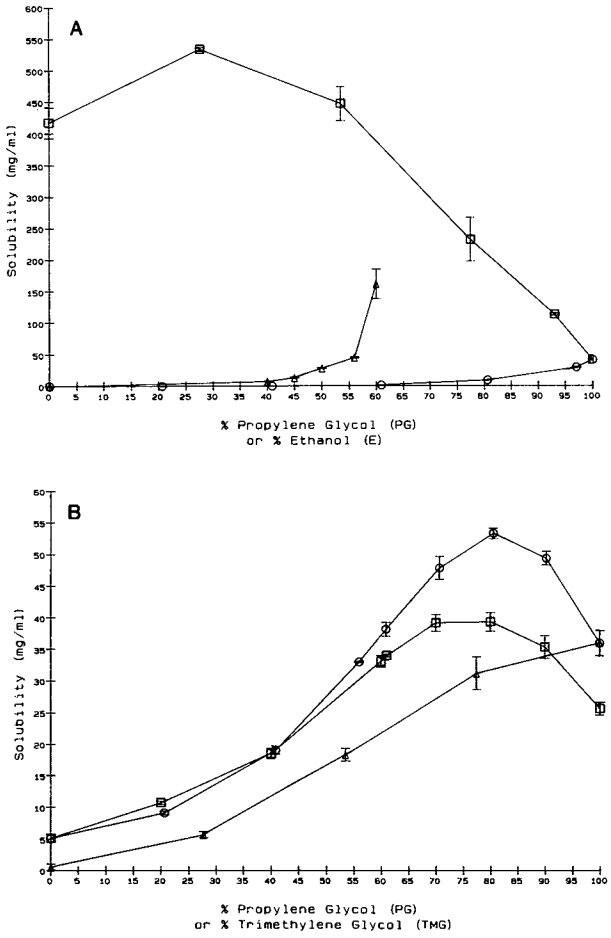

Fig. 2. (A) The solubility of nicardipine at 25°C in blends of (○) PG/W, (□) PG/LA, and (△) E/W. (B) The solubility of nicardipine hydrochloride at 25°C in blends of (○) PG/W, (□) TMG/W, and (△) PG/LA.

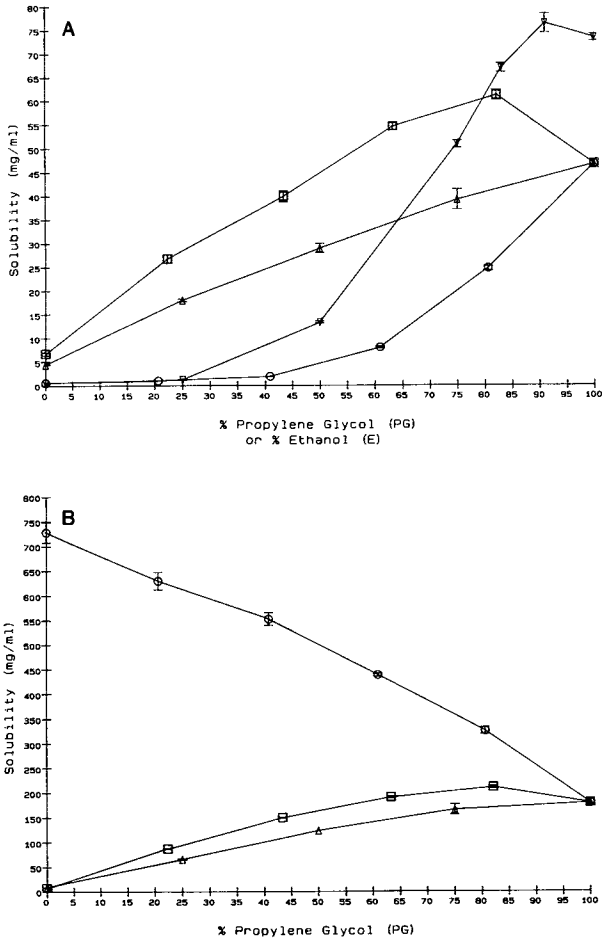

Fig. 3. (A) The solubility of ketorolac at 25°C in blends of (○) PG/W, (□) PG/LA, (△) PG/OA, and (▽) E/W. (B) The solubility of ketorolac tromethamine at 25°C in blends of (○) PG/W, (□) PG/LA, and (△) PG/OA.

Table II. Vehicle Blends Chosen for Percutaneous Absorption Testing in Rhesus Monkeys

Compound	Vehicle composition: Solvents	Vehicle composition: % (w/w)	Solubility (SD) (mg/ml)
Nicardipine	PG/W	97/3	29.0 (0.7)
	PG/LA	93/7	113 (1.6)
	PG/LA/W	90/7/3	99.2 (4.3)
	PG/W/AZ	87/3/10	87.3 (4.4)
	E/W[a]	56/44	45.0 (0.7)
Nicardipine hydrochloride	PG/W	56/44	33.0 (0.1)
	TMG/W	61/39	34.0 (0.2)
Ketorolac	PG/W	77/23	21.0[b]
	PG/LA	16/84	21.0[b]
	PG/OA	32/68	21.0[b]
	PG/W/TW	73/22/5	18.4 (0.2)
	E/W[a]	63/37	21.0[b]
Ketorolac tromethamine	PG/LA	7/93	29.8[b]
	PG/OA	8/92	29.8[b]

[a] Gelled with 1.5% hydroxypropyl cellulose (Klucel, HF; Hercules, Inc., Wilmington, Del.).
[b] Interpolated solubility value.

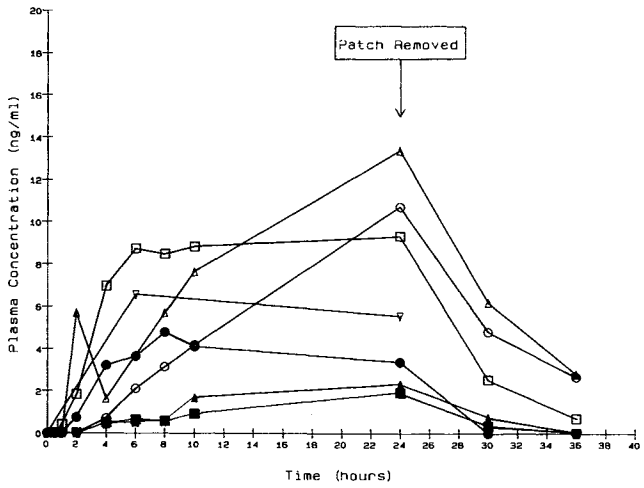

Fig. 4. Plasma levels of nicardipine and nicardipine hydrochloride following transdermal administration of nicardipine in (○) PG/W, (□) PG/LA, (△) PG/LA, (△) PG/LA/W (N = 2), (▽) PG/W/AZ (N = 2), and (●) E/W and nicardipine hydrochloride in (■) PG/W, and (▲) TMG/W.

Figures 2 and 3 show solubility profiles for nicardipine in PG/W, PG/LA, and E/W blends, nicardipine hydrochloride in PG/W, TMG/W, and PG/LA blends, ketorolac in PG/W, PG/LA, PG/OA, and E/W blends, and ketorolac tromethamine in PG/W, PG/LA, and PG/OA blends. These data did not correlate well with various theories predicting solubility in mixed solvent systems, including the regular solution theory and the extended Hildebrand solubility approach (21–23). Based on the results of the solubility studies, vehicle blends for formulations were chosen for percutaneous absorption testing (see Table II).

Nicardipine Absorption Studies. The percutaneous absorption of nicardipine and nicardipine hydrochloride formulations at 90% saturation was tested in rhesus monkeys. The results are shown in Fig. 4.

Both formulations of nicardipine hydrochloride gave much lower plasma levels than all of the formulations of nicardipine, indicating that the more lipophilic free base penetrates the skin much more readily than the hydrochloride salt. Plasma levels obtained from the TMG/W formulation of nicardipine hydrochloride were comparable to plasma levels obtained from the PG/W formulation, indicating that trimethylene glycol did not provide greater absorption enhancement compared with propylene glycol.

Plasma levels obtained from the PG/W formulation of the more lipophilic free base were roughly five times higher than plasma levels obtained from the PG/W formulation of the hydrochloride salt. With the PG/W formulation containing nicardipine, steady-state plasma levels had not been achieved by the time the HTCs were removed at 24 hr. In comparison, plasma concentrations obtained from the PG/LA, PG/W/AZ, and E/W formulations containing nicardipine achieved steady state after 6 hr. Linoleic acid, Azone, and ethanol, therefore, enhanced the initial rate of nicardipine absorption into the systemic circulation. In decreasing order of enhancement, LA > AZ > E. The PG/LA/W formulation of nicardipine provided a plasma concentration profile with characteristics associated with both linoleic acid (initial absorption rate enhancement) and the PG/W combination (gradual increase in plasma levels for 24 hr).

Assay results for the amount of compound remaining in the HTCs following percutaneous absorption studies are shown in Table III. The absolute bioavailability relative to an intravenous dose of nicardipine hydrochloride is also shown in Table III. The systemic availability of nicardipine and nicardipine hydrochloride from all of the formulations tested was low despite depletion of the HTCs of up to 57.5% of the loading dose. The highest absolute bioavailability obtained was from the PG/LA/W formulation of nicardipine (0.77% bioavailability).

Ketorolac Absorption Studies. The percutaneous absorption of ketorolac and ketorolac tromethamine in formulations at 95% saturation was tested in rhesus monkeys. The results are shown in Fig. 5. The PG/W, PG/W/TW, and E/W formulations containing ketorolac gave low plasma levels. Therefore, Tween 20 and ethanol did not provide greater enhancement of ketorolac penetration through the stratum corneum as compared to propylene glycol. The plasma level profile obtained from the PG/W formulation of ketorolac parallelled the plasma level profile obtained from the PG/W formulation of nicardipine, with plasma levels rising gradually throughout the 24-h period.

Plasma levels obtained from the PG/LA and PG/OA formulations of both ketorolac and ketorolac tromethamine

Table III. Absorption of 93 mg Nicardipine or 100 mg Nicardipine Hydrochloride Following 24-hr Transdermal Application in Rhesus Monkeys

Compound	Vehicle	C_{max} (SD) (ng/ml)	AUC (SD) (ng-hr/ml)[a]	Bioavailability (SD)[b]	% remaining (SD)
Nicardipine	PG/W	10.7 (0.40)	189 (35)	ND[c]	42.5 (2.9)
	PG/LA	9.33 (0.75)	233 (30)	ND	79.4 (7.7)
	PG/LA/W	13.4 (0.42)	272 (16)	0.77 (0.05)	50.4 (2.2)
	PG/W/AZ	6.55 (1.6)	128 (25)	0.37 (0.07)	49.8 (3.0)
	E/W	4.77 (2.6)	90.8 (40)	0.26 (0.11)	94.6 (2.1)
Nicardipine hydrochloride	PG/W	1.90 (2.4)	31.6 (46)	ND	88.2 (4.4)
	TMG/W	2.33 (0.23)	44.2 (13)	ND	104 (2.3)

[a] AUC values are approximate given the 14- to 18-hr interval between later time points.
[b] Bioavailability determined relative to i.v. bolus dosing.
[c] Not determined.

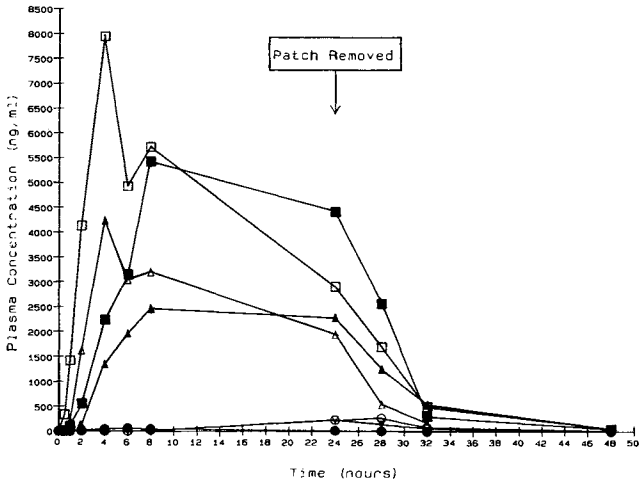

Fig. 5. Plasma levels of ketorolac and ketorolac tromethamine following transdermal administration of ketorolac in (○) PG/W, (□) PG/LA, (△) PG/OA, (▽) PG/W/TW, and (●) E/W and ketorolac tromethamine in (■) PG/LA and (▲) PG/OA.

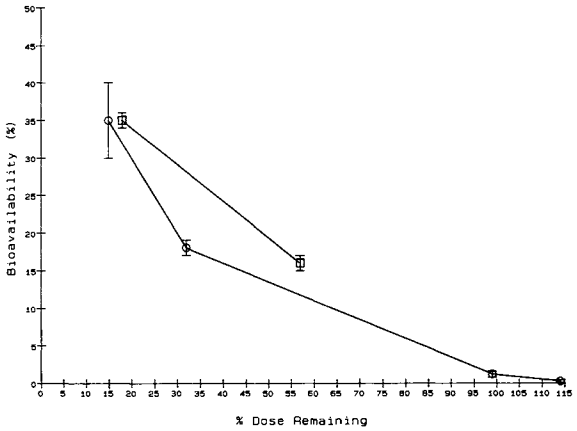

Fig. 6. Correlation between absolute bioavailability of ketorolac (○) and ketorolac tromethamine (□) administered using Hill Top Chambers and percentage dose remaining in the Hill Top Chambers after 24 hr.

were significantly higher. Both linoleic acid and oleic acid, in combination with propylene glycol, enhanced the penetration of both the free acid and the more hydrophilic tromethamine salt. The results of this study were consistent with the results obtained by Cooper (14). Enhancement obtained by using a blend of propylene glycol and a long-chain fatty acid was inversely proportional to the degree of saturation of the fatty acid hydrocarbon chain.

Assay results for the amount of compound remaining in the HTCs following percutaneous absorption studies are shown in Table IV. The absolute bioavailabilities of ketorolac and ketorolac tromethamine relative to an intravenous dose of ketorolac tromethamine are also shown in Table IV. The highest absolute bioavailability was achieved with the PG/LA formulations of both ketorolac and ketorolac tromethamine (35% bioavailability). The assay results for compound remaining in the HTCs correlated well with the bioavailability results, with the bioavailability inversely proportional to the percentage dose remaining (see Fig. 6).

Enhancement Effect of Vehicle Components. In summary, the choice of vehicle significantly affected the percutaneous absorption of nicardipine, nicardipine hydrochloride, ketorolac, and ketorolac tromethamine in the rhesus monkey. Formulations of compound in PG/W and TMG/W blends gave plasma levels of compound characterized by a gradual rise in plasma concentrations over 24 hr. Both trimethylene glycol and the nonionic surfactant Tween 20 had no additional enhancement effect. Azone and ethanol were both found to enhance plasma levels of nicardipine as well as to increase the initial flux of compound into the systemic circulation. Ethanol, however, did not enhance percutaneous absorption of ketorolac, indicating that absorption enhancement provided by ethanol varies by compound. Formulations containing oleic or linoleic acids, in combination with propylene glycol, were found to enhance compound plasma levels, with an increase in the initial flux of compound into the systemic circulation as well.

In general, higher fluxes were observed with the more lipophilic compounds nicardipine and ketorolac. The PG/LA ketorolac formulation and the PG/LA/W nicardipine formulation provided the highest absolute bioavailability for these compounds.

Table IV. Absorption of 60 mg Ketorolac or 85 mg Ketorolac Tromethamine Following 24-hr Transdermal Application in Rhesus Monkeys

Compound	Vehicle	C_{max} (SD) (μg/ml)	AUC (SD) (μg-hr/ml)[a]	Bioavailability (SD)[b]	% remaining (SD)
Ketorolac	PG/W	0.270 (0.17)	4.57 (1.7)	1.2 (0.4)	99.4 (3.0)
	PG/LA	7.94 (1.0)	126 (23)	35 (5)	14.9 (1.9)
	PG/OA	4.24 (1.2)	69.2 (6.7)	18 (1)	32.2 (4.6)
	PG/W/TW	0.239 (0.14)	3.87 (1.9)	ND[c]	94.5 (9.2)
	E/W	0.0617 (0.032)	1.11 (0.28)	0.3 (0.1)	114 (3.2)
Ketorolac tromethamine	PG/LA	5.42 (0.59)	121 (6.4)	35 (1)	17.6 (6.3)
	PG/OA	2.47 (0.46)	62.9 (2.4)	16 (1)	56.7 (7.4)

[a] AUC values are approximate given the 16-hr interval between later time points.
[b] Bioavailability determined relative to i.v. bolus dosing.
[c] Not determined.

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