Pharmaceutical Research, Vol. 7, No. 12, 1990

Report

Investigations on the Percutaneous Absorption of the Antidepressant Rolipram *in Vitro* and *in Vivo*

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Received January 31, 1990; accepted June 5, 1990

In vitro experiments using full-thickness human skin showed that it was feasible to deliver therapeutic amounts of the new antidepressant drug rolipram. Simple transdermal devices were constructed, and the presence of isopropyl myristate (IPM) in a silicone adhesive (Dow Corning X7-2920) enhanced the flux across excised human skin. The steady-state fluxes from adhesive mixtures containing 0, 5, and 10% IPM were 3, 5.2, and 6 $\mu g/cm^2/hr$, respectively. The *in vitro* experiments were confirmed in a clinical study involving six healthy male volunteers. The formulations tested were an alcoholic solution and adhesive patches containing 5 and 10% IPM. The dose of drug administered was 0.5 mg/cm^2 and the device size 25 cm^2. Blood samples were withdrawn over a 24-hr period and analyzed using radioimmunoassay. The topical applications were well tolerated, with only mild or no side effects. A lag time of approximately 2 hr was found for the detection of rolipram in the plasma (detection limit, 50 pg/ml). Interindividual variations both for the peak drug levels and throughout the delivery were quite high but this magnitude of variation has been observed in many other transdermal studies. Plasma levels between 1 and 2 ng/ml were found for all formulations and the AUC_{0-30hr} was significantly higher for the patch containing 5% IPM.

KEY WORDS: transdermal delivery; rolipram; isopropyl myristate; skin absorption; *in vitro–in vivo* correlations.

INTRODUCTION

Rolipram [4-(3-cyclopentyloxy-4-methoxy-phenyl)-2 pyrrolidone] is under clinical development as an antidepressant. Animal studies have shown (1–3) that rolipram selectively inhibits cAMP phosphodiesterase activity and thereby increases the synthesis and release of central noradrenaline. No effects were observed on dopaminergic or serotoninergic systems (4). Rolipram has been shown to be clinically effective in the treatment of endogenous depression (5–7). The anticipated therapeutic dose of 1 mg t.i.d. does not lead to any changes in vital signs and has been tolerated without major side effects by a large number of volunteers and patients. The drug shows a broad therapeutic range [tolerance limit (vomit), 15 mg t.i.d.].

The pharmacokinetics of rolipram has been investigated in animal species (8) and man (9). In humans, rolipram is completely absorbed after oral ingestion but absolute bioavailability is reduced and rather variable ($73 \pm 25\%$; $n = 6$) (9). The half-life of the terminal disposition phase of drug plasma levels ranged from 1.5 to 3.8 hr (9). Prior to excretion rolipram is completely metabolized and therefore the total clearance rate from plasma (2 ml/min/kg) is assumed to reflect the metabolic clearance rate (9).

The pharmacokinetics and anticipated clinical use of rolipram, i.e., rapid elimination from plasma, reduced and variable absolute bioavailability, low daily dose, and chronic treatment, are in favor of a transdermal route of administration. On the basis of clinical results and pharmacokinetic data it is anticipated that constant plasma levels of 2–3 ng/ml might reflect the levels required to achieve therapeutic effect.

Clinical phase I studies were carried out in order to check the feasibility of rolipram for transdermal administration (10). Applied as an alcoholic solution or as an aqueous system, about 8% of the dose of 0.2 mg/cm^2 spread over an area of 10 cm^2 was absorbed during 24 hr. Maximum plasma drug levels were in the range of 0.6–0.8 ng/ml. Increases in the dose applied were not accompanied, however, by plasma level elevations (10). This suggests that the thermodynamic activity of the rolipram remained constant despite the increase in the amount applied.

The aims of the present studies were to investigate *in vitro* the suitability of enhancers to increase the percutaneous flux of rolipram and to confirm preliminary *in vitro* findings by a clinical-pharmacokinetic study in healthy volunteers.

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0724-8741/90/1200-1307$06.00/0

MATERIALS AND METHODS

In Vitro Studies

The *in vitro* penetration rate of rolipram was determined using full-thickness excised human skin mounted in all-glass Franz-type diffusion cells. The skin was obtained postmortem from an abdominal site from Caucasian subjects, both male and female, varying in age from 63 to 72. Initial studies were conducted with an aqueous solution of rolipram. Due to solubility constraints, 25% ethanol was added to both the donor and the receptor phases. In addition, the receptor compartment was buffered to pH 7.4 (phosphate saline), stirred magnetically, and thermostated at 37°C. The donor phase contained 1 ml of 3.63 m*M* rolipram (^{14}C labeled, supplied by Schering) solution and was covered to minimize evaporation. The skin surface area was 2.1 cm^2 and had a surface temperature of 32°C. Experiments were conducted in triplicate, and samples removed regularly from the donor compartment and analyzed by scintillation counting.

To investigate potential transdermal systems, the rolipram was dispersed in an amine-resistant silicone adhesive (Dow Corning X7-2920, supplied as a gift). In addition to the adhesive, isopropyl myristate (IPM) was also present as a potential penetration enhancer. The IPM was obtained cosmetic grade from Croda Chemicals. Preparation of the adhesive mixture was as follows. The solvent in which the adhesive was supplied was allowed to evaporate. IPM plus 1 mg rolipram was dissolved with the adhesive (4.3 mg) in a small quantity of dichloromethane. This was applied to the surface of the skin in the Franz-type diffusion cell. Experiments were conducted in triplicate.

The skin had a surface temperature of 32°C, at which temperature the dichloromethane evaporated rapidly, to leave a film of adhesive plus drug 50 μm thick. The receptor phase, pH 7.4 phosphate-buffered saline containing 25% ethanol, was maintained at 37°C in a thermostated bath. The ethanol was present to ensure that the rolipram flux was not influenced by any solubility constraints in the receptor phase. Samples were removed from the receptor phase at regular time intervals over a 48-hr period, and the rolipram concentration was determined by scintillation counting.

CLINICAL STUDY

Materials

Formulations for topical rolipram application were either an ethanolic solution of 20 mg/ml (A) or patches containing 5% (w/w) IPM (B) and 10% (w/w) IMP (C). Patches were prepared freshly before each application using 362 mg dry silicone adhesive (Dow Corning X7-2908), 5 or 10% IPM (Croda, cosmetic grade), and 723 μl drug solution in chloroform (100 mg/ml). The ingredients were mixed and spread as an approximately 100-μm layer onto a backing polymer sheet (3M Health Care Ltd) using a wire wound bar to ensure reproducibility. Experiments to produce 50-μm-thick layers comparable to the adhesive thicknesses used in the *in vitro* studies were not successful. The dose of a 25-cm^2 patch was 12.5 mg rolipram. The concentration of rolipram in the patches was measured by extraction with dichloromethane and subsequent HPLC analysis. To test for local skin reactions, placebo patches were also prepared.

HPLC Analysis

An ODS II (5-μm) column was used with a mobile phase consisting of methanol:water (65:35). Using a flow rate of 1 ml/min rolipram eluted after 7 min and was detected at 230 nm. The system contained a guard column because of the presence of the adhesive; the areas under the peaks were integrated to give quantification of the rolipram.

Radioimmunoassay (RIA)

A specific RIA was used for estimation of drug plasma levels. The assay has been described previously (8). Using a t-butylether extract of 0.1 ml plasma the detection limit was 50 pg rolipram/ml.

Design of Clinical Study

The study had an open, randomized, crossover Latin square design. Six healthy male volunteers participated in the study (Table I) and received the three treatments (formulations A–C) at weekly intervals. Formulations were applied to premarked forearm skin areas of 5 × 5 cm (alternate arms at different test days). The alcoholic solution (625 μl) was applied to the skin area in five aliquots and covered with a plastic film after evaporation of the solvent. The test and placebo patches were applied to different forearms and each covered with a tubi grip for 24 hr. Thereafter patches and plastic films were removed and the skin area was cleaned by means of alcoholic swabs. Residues of formulations and alcoholic swabs were stored separately and kept at −20°C until analysis.

Before and 1, 2, 4, 6, 8, 10, 12, 14, and 24 hr after the start of each treatment, 5 ml blood was drawn from a peripheral arm vein and mixed with an anticoagulant, and plasma was prepared. Additional samples were obtained 1, 2, 4, and 6 hr after the end of treatment. The blood samples were taken from the placebo-treated arm. Plasma samples were kept deep frozen until analysis. Vital signs (systolic and diastolic blood pressure, heart rate) were recorded before the start of treatment, 2, 4, 8, 14, and 24 hr after dermal applications, and 6 hr after the end of treatment. Local skin reactions were assessed at the time of removal of the formulations.

Pre- and poststudy health screens covered a medical examination (including an ECG), the recording of medical history, and laboratory tests (blood haematology/biochemistry). Written informed consent was obtained from each volunteer and the study was approved by the local ethics committee.

Evaluation

In Vitro *Experiments*

Figure 1 shows typical results for the *in vitro* penetration of rolipram from an aqueous-ethanolic solution through full-thickness excised human skin. During the initial stages there is a characteristic lag phase followed by pseudo-steady-state diffusion. The steady state flux (J_{ss}) was calcu-

Table I. Personal Data for Volunteers and Details of Treatments[a]

	Volunteer No.					
	1	2	3	4	5	6
Initials	J.K.	P.H.	M.L.	M.M.	H.D.	O.M.
Sex	M	M	M	M	M	M
Age (years)	28	28	27	21	23	24
Weight (kg)	75	70	65	74	72	70
Height (cm)	180	188	174	170	183	180
1. Treatment (01/27/89)						
Formulation	B	B	A	C	C	A
Dose (mg)	14.8	14.8	12.5	13.8	13.8	12.5
LR	—	1	—	—	2	—
2. Treatment (02/03/89)						
Formulation	C	A	C	B	A	B
Dose (mg)	15.4	12.5	15.4	15.1	12.5	15.1
LR	—	4	5	4	—	3
3. Treatment (02/10/89)						
Formulation	A	C	B	A	B	C
Dose (mg)	12.5	13.0	15.1	12.5	15.1	13.0
LR	—	3	5	3	3	3

[a] Prestudy screen was on 01/10/89 or 01/12/89 and poststudy screen on 03/01/89.
Means and SD of age, weight, and height of volunteers were 25 ± 3 years, 71 ± 4 kg, and 179 ± 6 cm, respectively. LR, local reaction with "tingling" (1), slight itch (2), itch (3), slight erythema (4), and pallor (5). A, B, C—formulations as defined in the text.

lated by linear regression and was found to be 3.5 μg/cm²/hr. Using this information it is possible to calculate the plasma levels (c_p) that may be achieved during transdermal delivery. Estimates are obtained using the equation

$$C_p = \frac{J_{ss}A}{V_d k_{el}}$$

where A is the area of the device (25 cm²), V_d the volume of distribution, and k_{el} the elimination rate from the plasma. For rolipram, the pharmacokinetic profile is triphasic, with the β phase being the most significant. The constants associated with this phase may be estimated as 1 liter/kg (i.e., 70 liters for a 70-kg volunteer) and 0.46 hr^{-1}, respectively. The estimated plasma concentration would thus be 2.7 ng/ml, which is in the therapeutic range and therefore indicates that transdermal delivery is feasible.

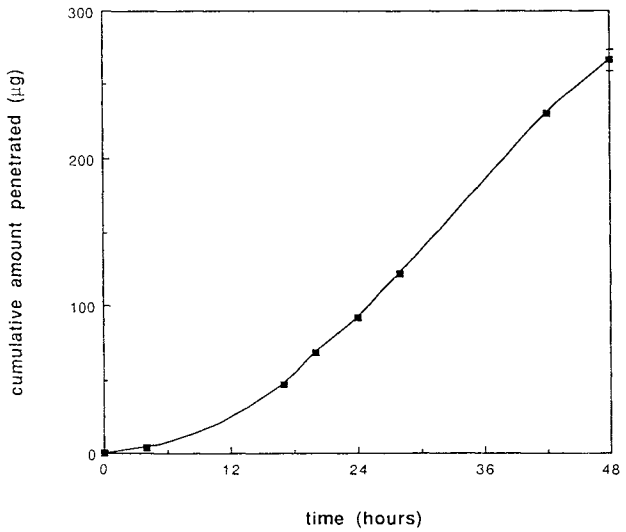

Fig. 1. The cumulative amount of rolipram penetrating full-thickness human skin. The results are the mean values from three experiments (±SE); where the error bars are not visible, they lie within the plotted data point.

During the experiments on the adhesive systems data were collected between 20 and 48 hr after the application of the formulations to the excised skin. They showed a linear increase in rolipram concentration with time, indicating that steady-state diffusion had been established. In assessing the feasibility of transdermal delivery, the steady-state flux was the most important factor to be considered, which is why little attention was given to the transient period before the maximum flux of drug was observed. The steady-state fluxes, as determined by linear regression, for the rolipram released across excised skin from the different adhesive preparations containing 0, 5, and 10% IPM were 3, 5.2, and 6 μg/cm²/hr, respectively. The latter two are higher than the fluxes produced from the solutions as the donor phase and confirm that these preparations have the potential for transdermal use. They also demonstrate the enhancement effects on the addition of IPM.

Clinical Study Evaluations

The rolipram plasma levels were calculated as nanograms per milliliter. Maximum concentrations (C_{max}) and times of attainment after the start of treatment (t_{max}) were assessed. The areas under the concentration vs time curve (AUC) were calculated from both 0 to 24 hr and 0 to 30 hr after the start of treatment (trapezoidal rule). The half-life of drug disappearance from the plasma was estimated from posttreatment samples by regression analysis ($\ell n\ c$ vs t). The AUC 0–24 hr and AUC 0–30 hr parameters were subjected to statistical analysis (analysis of variance with Duncan's mul-

tiple-range test for Latin square design). Other pharmacokinetic parameters were evaluated by descriptive statistics (means ± SD).

RESULTS

Clinical Study

All treatments were well tolerated. As judged by pre- and poststudy health screens and vital signs records, no drug-related systemic effects were observed. Local side effects (Table I) were either absent or mild in nature (itch, pallor, erythema). No dropouts or sampling irregularities occurred during the study.

In all six volunteers and after all three treatments, a lag time of about 2 hr was observed before the plasma levels exceeded the detection limit of 50 pg/ml. Thereafter drug plasma levels increased to variable maximum levels or steady-state concentrations. As shown in Fig. 2, interindividual variation in plasma levels was high for all formulations. Constant plasma levels of below 1 ng/ml were reached for the period of about 8–24 hr after application of the alcoholic solution (formulation A), whereas drug plasma levels of above 1.5 ng/ml were obtained for the same period after treatment with formulation B. A similar plasma level profile with constant levels of about 1.2 ng/ml was obtained for formulation C. A direct comparison of mean plasma levels is given in Fig. 3 for all three treatments.

Table 2 summarizes the pharmacokinetic parameters evaluated. Following application of formulation A, maximum plasma levels of 1.21 ± 0.8 ng/ml (range, 0.57–2.9 ng/ml) were measured 17 ± 8 hr (range, 8–25 hr) after start of treatment. The $AUC_{0-24\ hr}$ ranged from 10.5 to 46.4 ng · hr · ml^{-1}. Following the removal of formulation, the plasma levels declined with a half-life of 3.7 ± 1.7 hr. After treatment with formulations B and C average maximum levels increased to 1.82 ± 1.0 and 1.57 ± 0.8 ng/ml, respectively. Mean t_{max} values were shortened to 15 hr (B) or 10 hr (C) but large interindividual differences existed. In accordance with higher maximum plasma levels, the $AUC_{0-24\ hr}$ values were elevated to 36.3 ± 21 ng · hr · ml^{-1} (B) and 24.0 ± 11 ng · hr · ml^{-1} (C). Half-lives of posttreatment rolipram plasma level disposition were estimated to be about 3 hr (both formulations).

Statistical analysis of the pharmacokinetic parameters ($\alpha = 0.05$) showed that the $AUC_{0-24\ hr}$ was significantly higher after application of formulation B as compared to formulations A and C, but C was not significantly different from A. No significant temporal patterns were shown. At a lower significance level ($\alpha = 0.1$), the $AUC_{0-30\ hr}$ was significantly greater after application of formulation B as compared to formulation A. No carryover (period effect) was seen.

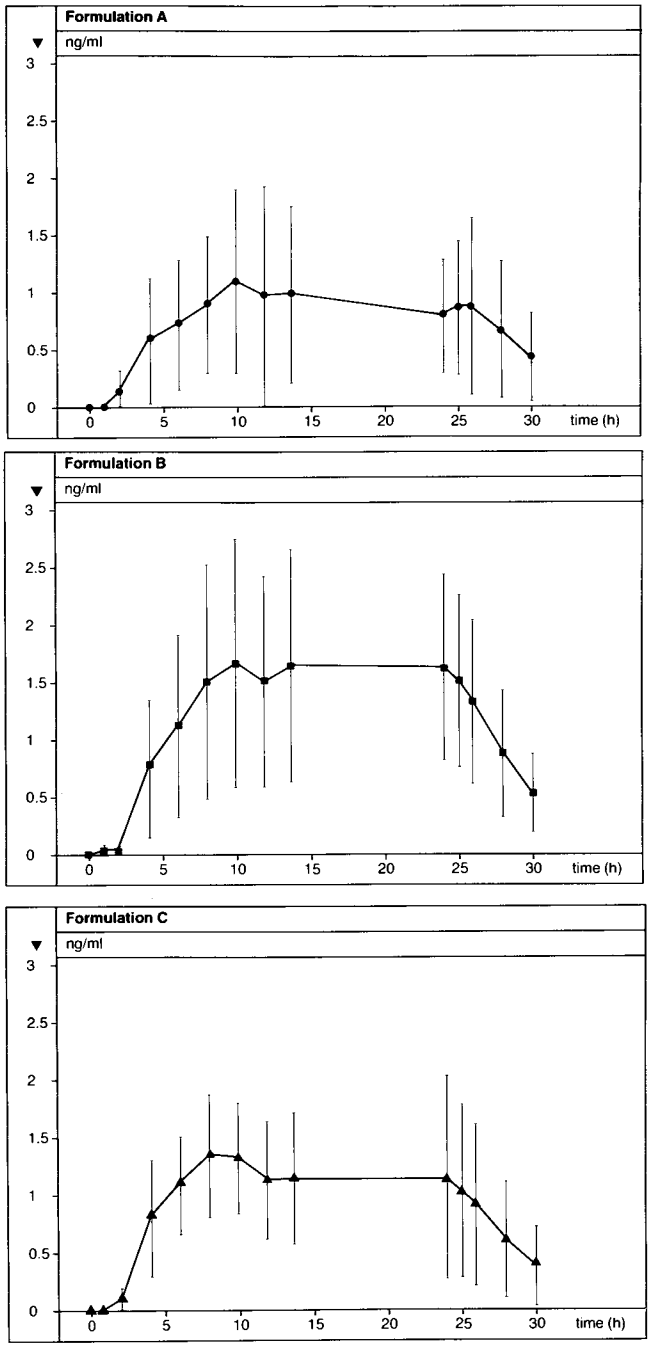

Fig. 2. Mean (±SD) rolipram plasma levels during and after 24-hr dermal application of 12.5–15.4 mg in six male volunteers. Drug was applied as an ethanolic solution (A) or as a patch containing either 5% (w/w) IPM (B) or 10% (w/w) IPM (C). The skin area was 25 cm^2.

DISCUSSION

In Vitro Experiments

The *in vitro* experiments showed that appreciable amounts of rolipram could be delivered across the skin using the simple adhesive formulations. The presence of IPM enhanced the steady-state flux but its inclusion modified the tack properties of the adhesive. When transdermal systems were synthesized, the tack property was considered satisfactory for the trial experiments; however, it is possible that changes may occur on storage. Since the pharmacokinetics of clearance are known for rolipram, it is possible to calculate the achievable blood levels given the steady-state input from the transdermal system. Assuming a plasma half-life of 1.5 hr and a volume distribution of 70 liters for a 70-kg individual, the steady-state plasma levels should be around 4

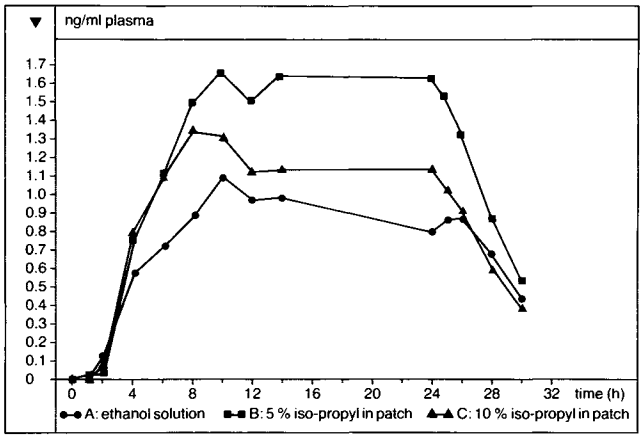

Fig. 3. Comparison of mean (n = 6) rolipram plasma levels as determined during and after a 24-hr dermal treatment of six male volunteers. Three formulations containing 12.5–15.4 mg drug were used in a randomized crossover design. The area of the forearm skin was 25 cm^2.

ng/ml. Since these are of the order required for therapeutic effect, it was considered appropriate to take these prototype transdermal systems into human volunteers to establish that levels approaching 4 ng/ml could be achieved.

Clinical Study

As expected, the dermal application of rolipram for all the three formulations was well tolerated. No systemic side effects and no or only mild local reactions were recorded. Local skin reactions were randomly distributed over placebo and test formulations (seven or eight recordings) and thereby assessed as not drug related.

Following all treatments drug plasma levels clearly exceeding the assay sensitivity were obtained. Independent of the formulation, there was an approximately 2-hr lag time until rolipram plasma levels became detectable. The steep increases of drug plasma levels thereafter clearly indicate that this is due to the passage of rolipram through the human skin. Approximately 8–10 hr after start of treatment steady-state conditions were reached and drug depletion from the plasma occurred only on patch removal. Using the data of Krause *et al.* (9), an intravenous injection of 0.1 mg (n = 6) resulted in an AUC of 12. 2 ng · hr · ml^{-1}. Comparing this number with the $AUC_{0-30\ hr}$ values of the present study, on average, 0.19, 0.31, and 0.23 mg rolipram were taken up via the skin after application of formulations A, B, and C, respectively. These amounts would reflect a transdermal flux of 317, 517, and 383 ng/cm^2/hr (formulations A–C, respectively). These are lower than the fluxes determined *in vitro*. There may be several reasons for this. First, the *in vitro* determinations were conduced using thinner films of adhesive (50 μm compared with 100 μm), and therefore the drug concentration in direct contact with the skin was higher *in vitro*. Second, in order to produce sink conditions the receptor phase of the *in vitro* experiment contained 25% ethanol; this may affect the permeability characteristics of the skin to rolipram. Third, it is difficult to extrapolate AUCs to give precise amounts of drugs absorbed. The $AUC_{0-24\ hr}$ values were different by factors of 5–7, and the total (metabolic) clearance rate of rolipram was previously reported to be highly variable: 0.94–3.8 ml/min/kg (9). Therefore the scatter in plasma levels found after dermal application of rolipram reflects variations of skin permeability and differences in metabolic drug handling.

Correlation of *in Vitro* and *in Vivo* Results

The *in vitro* experiments gave positive indications of the feasibility of transdermal delivery and these were confirmed *in vivo*. However, the plasma levels obtained *in vivo* were not as high as expected, although of the correct order of magnitude. Reasons for this have been discussed above. The *in vitro* data also demonstrate that the systems produced were capable of delivering drug over periods longer than 1 day. Since the observed plasma levels did not drop until after patch removal, it was not determined how long the patch would remain capable of sustaining steady levels.

In vitro the flux of rolipram was enhanced by the presence of IPM, with the system containing 10% IPM being optimal. *In vivo,* the 5% device was more efficient, although

Table II. Pharmacokinetic Parameters Calculated from Plasma Rolipram Levels in Six Volunteers Dermally Treated with Three Formulations for 24 hr in a Crossover Design[a]

	Volunteer No.																				
	1			2			3			4			5			6			Mean ± SD		
Parameter	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
C_{max} (ng/ml)	1.0	0.52	0.45	0.89	1.42	1.39	0.57	1.96	1.26	2.90	3.61	2.01	1.03	1.85	1.51	0.91	1.58	2.81	1.21 ± 0.8	1.82 ± 1.0	1.57 ± 0.8
t_{max} (hr)	10	10	6	25	24	10	24	8	6	12	10	8	8	14	8	25	24	24	17 ± 8	15 ± 7	10 ± 7
$AUC_{0-24\ hr}$ (ng hr ml^{-1})	11.0	10.3	6.4	14.7	27.7	24.0	10.5	41.6	20.3	46.4	73.2	28.7	15.6	39.4	24.5	13.1	25.6	39.8	18.5 ± 14	36.3 ± 21	24.0 ± 11
$AUC_{0-30\ hr}$ (ng hr ml^{-1})	12.7	10.6	7.5	19.0	28.6	28.0	12.7	42.7	23.1	57.5	76.1	32.4	17.5	40.7	28.7	17.8	27.4	51.3	22.9 ± 17	37.7 ± 22	28.5 ± 14
$T_{1/2}$ (hr)	3.0	2.0	3.5	5.0	2.0	2.5	1.6	2.3	2.3	3.3	4.0	4.0	n.c.[b]	2.3	2.3	5.8	4.5	4.0	3.7 ± 1.7	2.9 ± 1.1	3.1 ± 0.8

[a] For explanation see text and legend to Table I.
[b] Not calculated.

again there was no clear-cut distinction. The work highlights the problems in extrapolating *in vitro* data but also demonstrates that *in vitro* studies can provide useful guidelines for assessing the potential of transdermal delivery.

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