Percutaneous Absorption and Metabolism of Lonapalene in Psoriatic Skin

Paul A. Lehman,[1,3] Raymond V. Tomlinson,[2] Jerry I. Johnson,[3] John E. Olerud,[1] William A. Akers,[2] and Thomas J. Franz[3,4]

Received November 19, 1991; accepted January 26, 1992

The percutaneous absorption and metabolism of lonapalene (6-chloro-2,3-dimethoxynaphthalene-1,4-diol-diacetate; RS-43179), a topically effective 5-lipoxygenase inhibitor, has been measured in six subjects with stable plaque-type psoriasis of the lower extremities. Lonapalene readily penetrates psoriatic skin, is rapidly and completely metabolized, and is almost entirely excreted in the urine. Unexpectedly we observed a trend for thigh (T) plaque skin to be more permeable than lower leg (LL) plaque skin as measured by total absorption (T, 44.8 ± 13.4%; LL, 24.9 ± 12.6% applied dose excreted), peak plasma levels (T, 209 ± 107; LL, 146 ± 81 ng Eq/ml), and peak rate of urinary excretion (T, 591.7 ± 112.2; LL, 318.4 ± 143.9 μg Eq/hr). There were also differences in the metabolic profiles between the two sites as measured by the quantity and proportion of dealkylated and conjugated products excreted in the urine.

KEY WORDS: lonapalene; skin; psoriasis; percutaneous absorption; cutaneous metabolism.

INTRODUCTION

Lonapalene (6-chloro-2,3 dimethoxynaphthalene-1,4-diol-diacetate; RS-43179; Fig. 1) is a selective potent inhibitor of 5-lipoxygenase *in vitro* (1) and *in vivo* (2) and has been found to be effective in the treatment of psoriasis (3,4). Topical application of lonapalene to psoriatic plaques resulted in decreased levels of leukotriene B_4 (LTB_4) without affecting 12-hydroxyeicosatetraenoic acid (12-HETE) or arachidonic acid levels. It has no effect on DNA synthesis.

Arachidonic acid metabolites, especially the 5-lipoxygenase product LTB_4, may be important in the pathogenesis of psoriasis. Increased levels of LTB_4, LTC_4, LTD_4, and the 12-lipoxygenase product 12-HETE have been found in psoriatic plaques (5–7). In addition, topical application of LTB_4 to normal skin results in the formation of intraepidermal microabscesses (8) and stimulation of epidermal proliferation (9), and intradermal injection causes an infiltration of polymorphonuclear neutrophils and increased vascular permeability (10). Furthermore, oral administration of benoxaprofen, a known inhibitor of 5-lipoxygenase, markedly improves psoriasis (10).

Clinical studies comparing lonapalene to fluocinolone acetonide and vehicle have found lonapalene to be superior to vehicle and at least as effective as the steroid (3,4). No systemic adverse effects have been reported other than local irritation. Lassus and Forsstrom (4) found a good to excellent response in 89% of the patients treated with lonapalene after 4 weeks. The response was similar to that of a medium potency topical corticosteroid. Jansen *et al.* (3) reported local irritation requiring discontinuation of treatment in only 3 of 60 patients. Recently another topical 5-lipoxygenase inhibitor, R-68151 (Janssen Research Foundation, Beerse, Belgium), was evaluated in a double-blind vehicle-controlled study in psoriasis (11). For R-68151 27% of the patients treated achieved complete clearing or marked improvement of their disease, compared with only 8% of the control group.

The objectives of the present study of lonapalene were (i) to evaluate the extent of percutaneous absorption of lonapalene from a nonoccluded application of a 1.0% ointment in patients with psoriasis vulgaris, (ii) to study the routes and rate of excretion of lonapalene from human subjects, and (iii) to determine the pattern of urinary metabolites of lonapalene in humans. In addition, *in vitro* studies of lonapalene absorption were conducted to supplement the *in vivo* data.

MATERIALS AND METHODS

Lonapalene was provided by Syntex Laboratories as a 1.0% concentration in an ointment base consisting of white petrolatum, white wax, propylene carbonate, propylene glycol, and glyceryl monosterate. Sufficient [^{14}C]lonapalene was used to give a specific activity of approximately 10.0 μCi/g of ointment. Radiolabel purity was greater than 98% as determined by HPLC. Chemicals and reagents were obtained from J. T. Baker Chemical Co (Phillipsburg, NJ) unless otherwise noted.

In Vivo Study

Subjects. Six male subjects with plaque-type psoriasis vulgaris on their lower extremities were enrolled, with each providing informed written consent (Table I). All had been free of topical antipsoriatic medication (e.g., corticosteroids, tar) for at least 2 weeks and systemic antipsoriatic therapy for at least 4 weeks (e.g., methotrexate, UVL, PUVA) prior to entry into this study. Within 14 days prior to the start and at the completion of the study, each subject received a physical examination and routine laboratory tests and were found to be in good health.

The subjects were hospitalized for 24 hr and had the ointment applied to an involved plaque area(s) of psoriasis totaling 200 cm^2 located on either the thigh or the lower leg (Table I), without exposure to uninvolved skin. Doses were applied to the psoriatic sites by extrusion from syringes which were weighed before and after dosing to determine the exact weight delivered. Each dose was rubbed in with a fingercot to cover the marked areas uniformly. The fingercots were saved for isotope analysis. The target dose to the

[1] Division of Dermatology, School of Medicine, University of Washington, Seattle, Washington 98115.
[2] Syntex Research, Palo Alto, California 94304.
[3] Present address: Department of Dermatology, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, Arkansas 72205.
[4] To whom correspondence should be addressed.

0724-8741/92/0900-1145$06.50/0

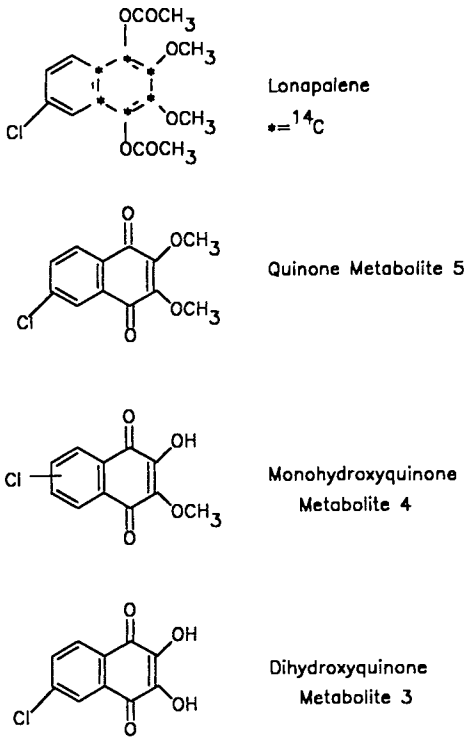

Fig. 1. Structure of lonapalene and its significant metabolites. Metabolite 2 (not shown) is a mixed pool of glucuronides of metabolites 3–5, which coelute as a single peak by HPLC. Metabolite 1 (not shown) is an unidentified metabolite with an early elution time near the void volume by HPLC and retains the radiolabel of the parent lonapalene. Identity numbers were assigned from the HPLC elution sequence.

200-cm^2 application area was 2 g of ointment containing approximately 20 μCi. The exact dose delivered to the skin was calculated from the gravimetric and radioactivity data (Table I). A protective thick plastic foam strip was taped around the edge of the dosed areas to prevent accidental contact with bedding or clothing.

Twelve hours after application, the dosed area was gently scraped with a metal spatula to remove the visible ointment remaining on the surface, following which the area was cleansed with gauze pads moistened with soapy water and rinsed. The sites were then covered with a clean gauze dressing overlaid with Saran Wrap. This wash and cover procedure was repeated at 24, 48, and 72 hr postdosing. The wash, gauze pads, and all items in contact with the dosed area were saved for analysis of radioactivity. The subjects were not allowed to shower or otherwise wet the application sites for 72 hr after dosing and were required to avoid sweat-producing activities and conditions during that period. The dosed area remained uncovered after the 72-hr wash.

Table I. Demographics and Dosing of Volunteers

Subject no.	Age (years)	Height (cm)	Weight (kg)	Application site	Dose applied[a]
1	47	176	81.6	Thigh	18.2
2	61	180	71.2	Thigh	16.6
3	31	178	109.3	Thigh	18.5
4	24	185	122.5	Lower leg	20.3
5	73	168	72.7	Lower leg	18.5
6	39	174	60.3	Lower leg	14.6

[a] Milligrams of lonapalene applied to 200 cm^2 from a 1% ointment.

Blood samples were collected in 15-ml heparinized tubes (Vacutainer) before drug application and at 2, 4, 8, 12, 24, 48, and 72 hr after dosing. Plasma was prepared by centrifugation and the samples were stored frozen until analyzed.

Complete urine collections were made in 4-hr pools at baseline and for the first 12 hr, followed by a 12- to 24-hr pool, and then as 24-hr collections thereafter to 120 hr. To prevent possible oxidation of samples, the urine specimens were made 0.1 *M* in ascorbic acid by adding an amount of 1 *M* ascorbic acid equal to 10% of the sample volume as soon as possible after collection. All samples were kept on ice until they could be frozen and stored.

Baseline stool samples were collected prior to applying the drug, then as provided daily through 120 hr after dosing. Samples were frozen as soon as possible after collection for storage.

Sample Analysis. Duplicate aliquots (0.5 ml) of plasma or urine were mixed with 10 ml of Biofluor and radioactivity was determined with a Packard Model 3330 Tri-Carb liquid scintillation spectrometer. When necessary, plasma aliquots were mixed with 0.2 ml of concentrated hydrogen peroxide to remove any color resulting from red-cell hemolysis.

Stool samples were homogenized in sufficient water to make a slurry, dried, and combusted in a Packard Sample Oxidizer, Model 306. The $^{14}CO_2$ recovery was determined by liquid scintillation counting.

Surface wash and covers were extracted in toluene to dissolve the petrolatum-based ointment and aliquots assayed by scintillation counting.

Lonapalene metabolites were isolated from urine and plasma by solid-phase extraction and high-pressure liquid chromatography (HPLC). BondElut columns (500 mg, 3 cm^3, Analytichem International) were prepared by acidification with dilute phosphoric acid (pH 2.5). The plasma or urine aliquots, adjusted to pH 2.5, were applied to the columns. After the columns were washed with water, they were eluted with methanol. Aliquots of the methanol solutions were reduced in volume by evaporating to near-dryness and reconstituted in HPLC mobile phase. A small volume (10–15 μl) of a standard mixture of lonapalene and metabolites 2–5 (Fig. 1) was mixed with each sample prior to analysis.

The extracted samples were filtered, injected onto a 5-μm Whatman Partisil ODS-3 column and eluted with 0.01 *M* citric acid (pH 2.5 with phosphoric acid):methanol (40:60, v/v) at a flow rate of 1 ml/min. Fractions (0.5 ml) were collected over 25 min and assayed for radioactivity by liquid scintillation spectrometry. Elution of the comixed standard was monitored by ultraviolet spectrophotometry at 229 nm.

Glucuronide metabolites were hydrolyzed after collecting the HPLC eluate glucuronide peak into ice-cold 0.1 *M* phosphate buffer made 0.1 *M* in ascorbic acid (pH 6.8), removing the methanol under a nitrogen stream, and adding 2000–4000 units of β-glucuronidase (Sigma Type IX from *Escherichia coli,* 800,000 units/g). The solution was incubated at 37°C for 1 hr. The activity of the enzyme under these conditions was verified by using phenolphthalein gluc-

uronide as a substrate and by detecting the hydrolyzed glucuronide colorimetrically at pH >8. After incubation, the study samples were extracted and analyzed by HPLC as described previously.

In Vitro Study

Lonapalene absorption was measured *in vitro* through cryopreserved dermatomed cadaver skin using Franz diffusion chambers (12). One-square centimeter sections from a single donor, five each from the thigh and abdominal regions, were cut and mounted on diffusion chambers containing phosphate-buffered saline (pH 7.3) with 0.5% Volpo (Croda Inc., New York). The receptor solution was maintained at 37°C and stirred magnetically at 600 rpm. Skin integrity was checked using a standard ^3H_2O permeation test (13) prior to lonapalene application and was found to be within normal limits.

Lonapalene ointment was applied by weight, using a glass rod, at a target dose equal to that used *in vivo* (10 mg/cm^2). The rod was weighed before and after application and the average delivered dose was found to be 9.5 mg/cm^2. The receptor solution was changed at intervals over the next 48 hr and the rate of drug absorption determined by gelling 1-ml aliquots with 5 ml scintillation fluid and counting in a liquid scintillation spectrometer. At 48 hr the skin surface was washed twice with 0.5 ml toluene, and the washes combined and assayed for radioactive content. Subsequently, the skin specimen was removed from the chamber, separated into epidermis and dermis, each dissolved in Soluene-350 (Packard Inst. Co., Downers Grove, IL), and analyzed for radioactive content.

RESULTS

The large area of involved skin (200 cm^2) needed for this study dictated the site of application. By chance, three of the volunteers had the required plaque(s) size on their lower leg and the other three volunteers on the midthigh. Although all samples were analyzed without knowledge of the site of application, as data became available it was found that the results were clearly dependent upon the site of application. For this reason the data have not been combined but, rather, are presented separately for thigh (T) and lower leg (LL).

In Vivo Study

Plasma. From a mean topical application of 17.8 ± 2.0 mg (mean ± SD) of lonapalene over 200 cm^2 (89 μg/cm^2), the plasma content of radioisotope rapidly achieved a maximum concentration at 8 hr in the three volunteers with thigh application and at 12 hr in the three volunteers receiving lower leg application (Fig. 2). The mean peak plasma concentration from the thigh application was 209 ± 83 ng Eq/ml and that on the lower leg was 146 ± 63 ng Eq/ml (Table II). Plasma half-life ($T_{1/2}$) as calculated between 12 and 48 hr averaged 7.3 ± 1.1 hr (T) and 8.0 ± 0.3 hr (LL). The area under the curve (AUC) 0–72 hr was 3.9 ± 2.0 μg Eq-hr/ml (T) and 3.0 ± 1.5 μg Eq-hr/ml (LL). Better than 75% of the 0–72 hr AUC value was contained in the first 24-hr period after application.

Urine. The rate of excretion of radioisotope into the urine is presented in Fig. 2 and Table III. Radioisotope excretion was greater from those volunteers receiving thigh applications than from those receiving lower leg applications. However, due to the small number of subjects, the regional differences are not statistically significant (maximum differences at 4 and 6 hr for plasma and at 10 hr for urine were found to be $0.1 > P > 0.05$ by student's t test). On average, 20% of the dose was excreted prior to washing off the ointment at 12 hr. Subsequently, another 13% of the dose was recovered by 72 hr. Total recovery in the urine averaged 33% of the dose (Table IV).

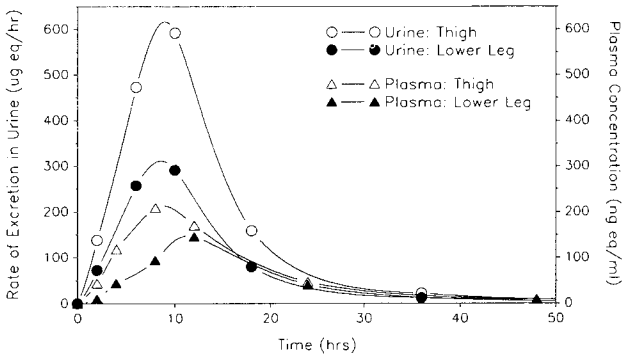

Fig. 2. Average plasma concentration (right axis) and average urinary rate of excretion (left axis) of radiolabel from the topical application of ^{14}C-lonapalene to the thigh (n = 3) and lower leg (n = 3) psoriatic plaques *in vivo*. Error bars excluded for clarity (see Tables II and III).

Stool. Total recovery of radioisotope in the stool was very low, amounting to only 2% of the dose (Table IV). No further analysis was performed on the stool samples.

Surface Washes and Protective Covers. The first surface wash and 24-hr protective dressing recovered nearly 40% of the applied dose (Table IV). However, only an additional 1% of the dose was found in the subsequent surface washes and dressings.

Metabolites. By HPLC, no parent lonapalene was detected in plasma or urine. The predominant metabolites in plasma were determined to be a mixed pool of glucuronide conjugates (metabolite 2) of the monohydroxy quinone (metabolite 4) and quinone (metabolite 5), which coelute by the HPLC method as a single peak but were resolvable after β-glucuronidase treatment. Less than 12% of the radioactivity found in the plasma was other aglycone metabolites (Table V), however, 39% of the radioactivity was not retained by the pre-HPLC BondElut column sample preparation step.

Nearly 91% of the radioactivity in the urine was found

Table II. Plasma Concentration (ng Eq/ml) of Lonapalene

Thigh[a]	Lower leg[a]	Time (hr)
0	0	0
44 ± 17	10 ± 60	2
118 ± 45	44 ± 17	4
209 ± 83	95 ± 37	6
171 ± 63	146 ± 63	12
47 ± 21	41 ± 12	24
10 ± 1	9 ± 3	48
6 ± 2	5 ± 2	72

[a] Average ± SD; n = 3.

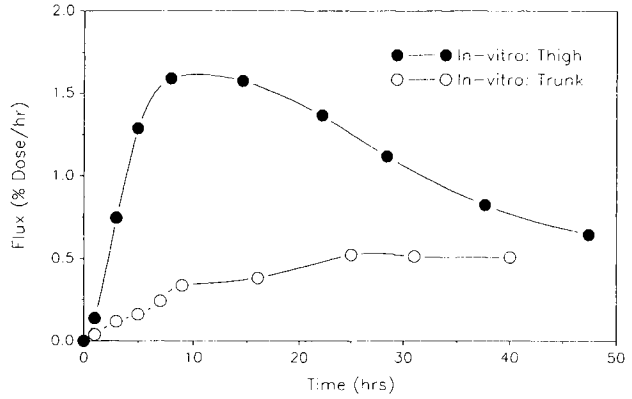

Fig. 3. Rate of penetration (flux) of lonapaline through human skin *in vitro*.

to coelute with the HPLC metabolite standards. Again, the major metabolite fraction was associated with the glucuronide conjugate pool as observed in the plasma. In addition, substantial amounts of the free unconjugated metabolites 3–5 were also detected (Table V) along with an unidentified metabolite which eluted very close to the solvent front (metabolite 1). After enzyme treatment of the glucuronide peak it was seen that there was a 1:8:14 ratio of metabolites 3, 4, and 5.

Segregating the urine metabolite data into the two application site groups (Table V), there were insignificant quantitative differences in the appearance of metabolite 1 and total glucuronides 2. However, there was a distinct difference in the appearance of metabolites 3 (dihydroxyquinone), 4 (monohydroxyquinone), and 5 (quinone) with site of application. The urine from the subjects receiving the ointment to the thigh demonstrated the presence of (unconjugated) metabolites 3 and 4, with no detectable levels of metabolite 5. Conversely those volunteers receiving the lower leg application excreted metabolite 5 ($P < 0.1$) but undetectable amounts of metabolite 3 ($P < 0.1$) and 4 ($P < 0.2$). Enzymatic treatment of the glucuronides showed a similar but less distinct distribution of aglycone metabolites to the unconjugated metabolites: more 3 and 4 from thigh application but more 5 from the lower leg application.

In Vitro Study

The percutaneous absorption of lonapalene was also studied *in vitro* in order to supplement the data obtained *in vivo*. The major difference between the two sets of experiments was the absence of a 12-hr surface wash *in vitro* and the use of abdominal skin as the second site (rather than lower leg). The results are presented in Table VI and Fig. 3.

Table III. Urinary Rate of Excretion (μg Eq/hr) of Lonapalene

Thigh[a]	Lower leg[a]	Time (hr)
0	0	0
138.4 ± 73.4	37.7 ± 40.1	2
472.8 ± 197.0	180.4 ± 178.9	6
591.7 ± 112.2	318.4 ± 143.9	10
158.9 ± 41.1	114.2 ± 5.4	18
22.0 ± 4.8	17.2 ± 5.1	36
2.2 ± 0.1	2.5 ± 1.2	60
0.7 ± 0.1	1.0 ± 0.5	84

[a] Average ± SD; $n = 3$.

Table IV. Total Recovery of Radioactivity as Percentage of Applied Dose

Specimen source	Thigh[a]	Lower leg[a]
Urine[b]	41.7 ± 11.1	23.9 ± 12.6
Feces[b]	3.1 ± 2.6	1.0 ± 0.1
12-hr wash + dressing[c]	29.8 ± 10.5	50.0 ± 29.0
24-hr wash + dressing	1.2 ± 1.0	1.4 ± 0.3
48-hr wash + dressing	0.1 ± 0.1	0.2 ± 0.1
Total recovery	75.7 ± 2.7	76.1 ± 28.0

[a] Average ± SD; $n = 3$.
[b] Total excretion over 120 hr after drug application.
[c] Dressing which had covered the application site prior to the next scheduled wash.

In agreement with the results obtained *in vivo*, lonapalene was found to be well absorbed and displayed a distinct regional variation in its absorption profile. Total absorption through thigh skin (the sum of receptor and dermal radioactivity) was 60.4%, reasonably close to the 44.8% seen *in vivo*. The difference between the two is directionally appropriate considering the absence of a 12-hr surface wash *in vitro*. Likewise, the peak rate of absorption of 1.5 μg/cm²/hr, seen at 8 hr *in vitro*, is close to the peak rate of excretion of 3.0 μg-Eq/cm²/hr (600 μg-Eq/200 cm²/hr) seen at 10 hr *in vivo*.

Ninety-one percent of the applied dose could be accounted for *in vitro*. Most of the unabsorbed material remained on the surface of the skin and was recovered in the surface wash at 48 hr. Epidermal content, presumed to be mostly stratum corneum content, was only 2.1 and 5.9% of the dose for abdominal and thigh skin, respectively. The lower content of abdominal skin correlates with its lower permeability to lonapalene, as would be expected from diffusion theory.

DISCUSSION

Lonapalene readily penetrates psoriatic skin, is rapidly and completely metabolized, and is eliminated predominantly in the urine. In contrast to many clinically useful drugs, such as the corticosteroids (14) and retinoids (15), where only 1–2% of the applied dose is absorbed through normal skin on most body sites, lonapalene can be ranked with compounds such as dinitrochlorobenzene, p-aminobenzoic acid, and salicylic acid, to which the skin is highly permeable (14).

Regional variation in percutaneous absorption is well recognized (16–19). Specifically, we have found that lower leg skin is more permeable than thigh skin to water and benzoic acid (20). In contrast, we observed a trend for just the opposite in this study: Psoriatic plaques on the thigh appeared more permeable than those on the lower leg. This is consistent with the observations made by Stoughton (21) that psoriatic lesions on the upper thighs respond better to topical glucocorticoids than lesions on the lower legs. The

Table V. Distribution of Metabolites in Plasma and Urine as Percentage of Recovery

Metabolite[a]	Urine[b]		Plasma[c]	
	Thigh[d]	Lower leg	Thigh	Lower leg
1	20.6 ± 5.5	21.6 ± 3.8	3.8 ± 6.6	5.9 ± 10.2
2	43.4 ± 8.1	47.1 ± 6.4	41.9 ± 20.1	48.4 ± 8.6
3	17.0 ± 15.6	0	1.7 ± 2.9	3.0 ± 5.1
4	12.7 ± 22.1	0	2.2 ± 3.8	0
5	0	14.7 ± 8.7	0	0
R[e]	6.3 ± 3.9	16.5 ± 3.5	34.6 ± 7.0	38.9 ± 11.1
Aglycone products of metabolite 2[f] from urine				
3	6.5 ± 10.4	0.6 ± 1.0		
4	30.0 ± 8.1	22.2 ± 7.5		
5	39.1 ± 11.1	54.2 ± 13.5		
R	15.0 ± 4.1	22.8 ± 8.2		

[a] Refer to Fig. 1 for metabolite structure identification.
[b] Urine collected from 0 to 24 hr.
[c] Plasma collected from 0 to 12 hr.
[d] Average ± SD; $n = 3$.
[e] Percentage of radioactivity not retained by the BondElut column during sample preparation (see Materials and Methods).
[f] Second HPLC separation of the products from β-glucuronidase treatment of the isolated glucuronide metabolites 2.

evolution of the psoriatic plaque must be directing the stratum corneum permeability characteristics independently of the normal regional development.

In spite of the fact that psoriatic plaques have characteristics that would indicate an altered barrier function, such as thickness (22), increased transepidermal water loss, and decreased water binding (23), as well as other physiological differences (24), distinct plaque permeability comparisons to uninvolved or normal skin have been limited and do not resolve this issue. Anthralin has been reported to be more permeable in psoriatic skin (25), yet hydrocortisone penetration was not different between psoriatic and normal skin (26).

This study was not designed to address the permeability differences between psoriatic and normal skin. However, the *in vitro* penetration of lonapalene from cryopreserved normal skin achieved only one-half the peak flux observed from the *in vivo* urinary excretion rate, suggesting that the psoriatic plaque may be more permeable than normal skin.

The *in vitro* study was performed primarily to ensure that the low mass balance observed *in vivo* [75% total recovery; typical for this type of study design (27)] was not a consequence of binding of lonapalene in the skin. (Although elimination of a volatile metabolite via the lungs cannot be ruled out, it seems unlikely considering the distribution of the radiolabel within the parent structure.) As better than 91% of the dose was recovered from the *in vitro* study, with very little of the lonapalene observed to be resident in the skin, the low *in vivo* mass balance is most likely a result of poor recovery of the exfoliated stratum corneum from the gauze site covers or inadvertent removal by the subjects during the topical exposure period in spite of their careful instruction and site protection. The latter may explain the greater variability observed in the mean total recovery from the lower leg sites (Table IV), where the first surface wash from volunteer No. 5 was only 20% of the applied dose, considerably lower than all the others. If this one value is excluded from the calculation of the total recovery from the lower leg, the standard deviation lowers to 0.1%. It should not be inferred that, because this volunteer's dose recovery was low, his total topical exposure was also lower than the others and a bias to the group; in fact, his kinetic and metabolite data were either the median or the high value for the leg group.

Table VI. Distribution and Flux of Lonapalene from *in Vitro* Study

Source	Trunk ($n = 5$)[a]	Thigh ($n = 6$)
Peak flux	0.5 ± 0.1	1.6 ± 0.7
Receptor solution[b]	16.9 ± 3.1	55.3 ± 14.3
Dermis[c]	1.5 ± 0.2	5.1 ± 2.1
Epidermis[c]	2.1 ± 0.4	5.9 ± 1.3
Surface[c]	70.6 ± 4.8	24.7 ± 10.6
Total recovery	91.0 ± 4.3	91.1 ± 12.6

[a] Values are mean % dose ± SD except flux, which is % dose/hr/cm^2.
[b] Cumulative penetration over 48 hr.
[c] Contents determined 48 hr after application.

The metabolic capacity of skin is well recognized (28) and has been characterized by *in vitro* organ skin culture (29), fresh excised skin (30), and enzyme induction *in vivo* (31). However, regional variation in cutaneous metabolism has received very little attention. The conversion of testosterone to dehydrotestosterone and other 5α reduced steroids is greater in skin from the scalp than the back (32) and greater in skin from sex organ regions of the body over trunk regions (33). More recently it has been reported that cutaneous metabolism of transdermal nitroglycerin was greater through less permeable abdomen skin than in the more per-

meable breast skin tested (34). Such distinctions may be attributed to differences in rate of penetration, enzyme distribution and capacity, or quantity of epidermal appendages (glands or hair follicles). In addition, the metabolic abilities unique to psoriatic plaques are contested. The decreased epidermal aryl hydrocarbon hydroxylase (AHH) activity and its poor inducibility in psoriatic plaques as compared to uninvolved skin reported by Chapman and Shuster (35,36) were not observed by Bickers *et al.* (37), who reported no difference in AHH enzyme activity and greater induction of AHH in plaque over uninvolved skin.

Cutaneous metabolism of lonapalene demonstrated regional variation in the metabolite profiles measured in the urine. If all metabolism of lonapalene were exclusively systemic, no difference would have been observed between the two sites of application. In reflection, systemic metabolism may well have moderated an even greater difference than that observed, a consequence of not being able to measure metabolite formation *in situ* at the dermal level. The multitude of pathways possible for the sequence of dealkylation and conjugation of lonapalene cannot be differentiated by this study. Nevertheless, the differences in abundance of metabolites 3–5, as both free and conjugated products, suggest a difference in metabolic activity between the leg and the thigh of psoriatic skin.

Lonapalene may become a very useful adjunct for the treatment of psoriasis. Its utility as a model compound for the study of skin permeation and metabolism is evident from this study. Further work on psoriatic skin, both involved and uninvolved, is warranted to clarify and confirm the site variations in both permeability and metabolic activity observed with lonapalene. Advancements in our understanding of permeability pharmacokinetics may well help explain the differences in efficacy of the various topical drugs used in the treatment of psoriasis.

REFERENCES

1. D. V. K. Murthy, M. Kruseman-Aretz, S. Rouhafza-Fard, C. J. Bedord, J. M. Young, G. Jones, and M. Venuti. Selective inhibitors of arachidonic acid (AA) 5-lipoxygenase by a novel antipsoriatic agent 6-chlorol,4-,-diacetoxy-2,3-dimethoxynapthalene (RS 43179). *Fed. Proc.* 44:886 (1985).
2. A. K. Black, R. D. R. Camp, A. I. Mallet, F. M. Cunningham, M. Hofbauer, and M. W. Greaves. Pharmacologic and clinical effects of lonapalene (RS 43179), a 5-lipoxygenase inhibitor, in psoriasis. *J. Invest. Dermatol.* 95:50–54 (1990).
3. C. T. Jansen, K. Lammintausta, R. E. S. Bullingham, and S. Forsstrom. A clinical trial of lonapalene, fluocinolone acetonide and vehicle in psoriasis. *J. Invest. Dermatol* 86:483 (1986).
4. A. Lassus and S. Forsstrom. A dimethoxynapthalene derivative (RS 43179 gel) compared with 0.025% Fluocinolone acetonide gel in the treatment of psoriasis. *Br. J. Dermatol.* 113:103–106 (1985).
5. K. Fogh, T. Herlin, and K. Kragballe. Eicosanoids in acute and chronic psoriatic lesions: Leukotriene B_4, but not 12-hydroxyeicosatetraenoic acid, is present in biologically active amounts in acute guttate lesions. *J. Invest. Dermatol.* 92:837–841 (1989).
6. E. A. Duell, C. N. Ellis, and J. J. Voorhees. Determination of 5, 12, and 15-lipoxygenase products in keratomed biopsies of normal and psoriatic skin. *J. Invest. Dermatol.* 91:446–450 (1988).
7. S. D. Brain, R. D. R. Camp, A. K. Black, P. M. Dowd, M. W. Greaves, A. W. Ford-Hutchinson, and S. Charleston. Leukotrienes C_4 and D_4 in psoriatic skin lesions. *Prostaglandins* 29:611–619 (1985).
8. R. Camp, R. Russel Jones, S. Brain, P. Woollard, and M. Breaves. Production of intraepidermal microabscesses by topical application of leukotriene B_4. *J. Invest. Dermatol.* 82:202–204 (1984).
9. F. W. Bauer, P. C. M. van de Kerkhof, and R. M. Maassen-De Grood. Epidermal hyperproliferation following the induction of microabscesses by leukotriene B_4. *Br. J. Dermatol.* 115:409–412 (1986).
10. J. J. Voorhees. Leukotrienes and other lipoxygenase products in the pathogenesis and therapy of psoriasis and other dermatoses. *Arch. Dermatol.* 119:541–547 (1982).
11. H. Degrees, P. Dockx, P. DeDoncker, K. DeBeule and G. Cauwenbergh. A double-blind vehicle-controlled study of R 68151 in psoriasis: A topical 5-lipoxygenase inhibitor. *J. Am. Acad. Dermatol.* 22:751–755 (1990)
12. T. J. Franz. Percutaneous absorption: on the relevance of in-vitro data. *J. Invest. Dermatol.* 64:190–195 (1975)
13. T. J. Franz and P. A. Lehman. The use of water permeability as a means of validation for skin integrity in *in vitro* percutaneous absorption studies. *J. Invest. Dermatol.* 94(4):525 (1990).
14. T. J. Franz. Kinetics of cutaneous drug penetration. *Int. J Dermatol.* 22(9):499–505 (1983).
15. T. J. Franz and P. A. Lehman. Systemic absorption of retinoic acid. *J. Toxicol.–Cut. Ocul. Toxicol.* 8(4):1–10 (1989).
16. R. J. Feldmann and H. I. Maibach. Regional variation in percutaneous penetration of ^{14}C cortisol in man. *J. Invest. Dermatol.* 48(2):181–183 (1967)
17. H. I. Maibach, R. J. Feldmann, T. Milby, and W. Serat. Regional variation in percutaneous penetration in man. *Arch. Environ. Health* 23:208–211 (1971)
18. F. J. Marzulli. Barriers to skin penetration. *J. Invest. Dermatol.* 39:387–393 (1962).
19. A. Rougier and C. Lotte. Correlations between horny layer concentration and percutaneous absorption. *Pharmacol. Skin* 1:81–102 (1987).
20. D. M. Lantz, P. A. Lehman, and T. J. Franz. Regional variation in percutaneous absorption. *J. Invest. Dermatol.* 92(3):466 (1989).
21. R. B. Stoughton. Percutaneous absorption of drugs. *Annu. Rev. Pharmacol. Toxicol.* 29:55–69 (1989).
22. J. Serup. Non-invasive quantification of psoriasis plaques-measurements of skin thickness with 15 mHz pulsed ultrasounds. *Clin. Exp. Dermatol.* 9:502–508 (1984).
23. H. Tagami, Y. Kanamaru, K Inoue, S. Suehisa, F. Inoue, K. Iwatsuki, K. Yoshikuni, and M. Yamada. Water sorption-desorption test of the skin *in vivo* for functional assessment of the stratum corneum. *J. Invest. Dermatol.* 78:425–428 (1982).
24. E. Berardesca and H. I. Maibach. Noninvasive bioengineering assessment of psoriasis. *Int. J. Dermatol.* 28(3):157–160 (1989).
25. J. C. T. Wang, B. G. Patel, C. W. Ehmann, and N. Lowe. The release and percutaneous permeation of anthralin products, using clinically involved and uninvolved psoriatic skin. *J. Am. Acad. Dermatol.* 16:812–821 (1987).
26. R. C. Wester, D. A. W. Bucks, and H. I. Maibach. *In vivo* percutaneous absorption of hydrocortisone in psoriatic patients and normal volunteers. *J. Am. Acad. Dermatol.* 8:645–647 (1983).
27. T. J. Franz, P. A. Lehman, and L. McGuire: *In vivo* methods for the assessment of percutaneous absorption in man. In J. Zatz (ed.), *Percutaneous Absorption,* Allured Wheaton, IL, 1992.
28. P. K. Noonan and R. C. Wester. Cutaneous metabolism of xenobiotics. In R. L. Bronaugh and H. I. Maibach (eds.), *Percutaneous Absorption,* 2nd ed., Marcel Dekker, New York, 1989, pp. 53–75.
29. M. Pham, J. Magdalou, G. Siest, M. Lenoir, B. A. Bernard, J. Jamoulle, and B. Shroot. Reconstituted epidermis: A novel model for the study of drug metabolism in human epidermis. *J. Invest. Dermatol.* 94:749–752 (1990).
30. J. E. Storm, S. W. Collier, R. F. Stewart, and R. L. Bronaugh. Metabolism of xenobiotics during percutaneous penetration:

Role of absorption rate and cutaneous enzyme activity. *Fund. Appl. Toxicol.* **15**:132–141 (1990).

31. W. A. Khan, S. S. Park, H. V. Gelboin, D. R. Bickers, and H. Mukhtar. Monoclonal antibodies directed characterization of epidermal and hepatic cytochrome P-450 isozymes induced by skin application of therapeutic crude coal tar. *J. Invest. Dermatol.* **93**:40–45 (1989).
32. M. E. Stewart, P. E. Pochi, J. S. Strauss, H. H. Wotiz, and S. J. Clark. *In vitro* metabolism of [^{3}H]testosterone by scalp and back skin: Conversion of testosterone into 5-androstane-3β,17β-diol. *J. Endocr.* 72:385–390 (1977).
33. J. D. Wilson and J. D. Walker. The conversion of testosterone to 5-androstan-17β-ol-3-one (diydrotestosterone) by skin slices of man. *J. Clin. Invest.* 48:371–379 (1969).
34. G. Santus, N. Watari, R. S. Hinz, L. Z. Benet, and R. H. Guy. Cutaneous metabolism of transdermally delivered nitroglycerin *in vitro*. *Pharmacol. Skin* **1**:240–244 (1987).
35. P. H. Chapman, M. D. Rawlins, and S. Shuster. Activity of aryl hydrocarbon hydroxylase in psoriatic skin. *Lancet* **1**:297–298 (1979).
36. S. Shuster, M. D. Rawlins, P. H. Chapman, and S. Rogers. Decreased epidermal aryl hydrocarbon hydroxylase and localized pustular psoriasis. *Br. J. Dermatol.* **103**:23–26 (1980).
37. D. Bickers, H. Mukhtar, T. Dutia-Choudhury, C. Marcelo, and J. Voorhees. Epidermal drug metabolism in human subjects: Comparative activity in normal individuals and patients with psoriasis. *Clin. Res.* **30**(2):260A (1982).