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Research Article

Percutaneous and Systemic Disposition of Hexamethylene Lauramide and Its Penetration Enhancement Effect on Hydrocortisone in a Rat Sandwich Skin-Flap Model

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The percutaneous absorption and distribution profile of hexamethylene lauramide (hexahydro-1-lauroyl-1H-azepine) were examined using a rat skin-flap model. After a topical dose to the skin flap, the drug concentrations in the vasculature at the site of drug application and in the systemic blood were monitored simultaneously. Hexamethylene lauramide penetrated the skin and reached a steady state in stratum corneum, viable epidermis, dermis, and cutaneous blood in 3 hr. Its concentration in the skin was much higher than that in the blood. Its apparent concentration in the epidermis was 19 times that in the dermis and about 3000 times that in the cutaneous blood. The percutaneous absorption of ^{14}C-hexamethylene lauramide resulted in ascending systemic blood concentrations throughout the experimental period, whereas the cutaneous blood levels remained steady. The topically absorbed hexamethylene lauramide was quantitatively recovered in urine (85%) and feces (13%). The half-lives of urinary and fecal excretion of ^{14}C-hexamethylene lauramide were 17 and 30 hr, respectively. Hexamethylene lauramide, when topically coadministered in an experimental formulation, enhanced the skin penetration of hydrocortisone with increased drug contents in the stratum corneum (2-fold) and with increased hydrocortisone concentrations in the cutaneous blood (3.4-fold) and the systemic blood (3.5-fold). The results indicated that the high concentration and retention of hexamethylene lauramide in stratum corneum and viable epidermis may contribute to its penetration enhancement effect in the skin. A steady state in percutaneous tissues was observed before the drug reached distribution equilibrium systemically. The systemic blood concentration of a topically applied agent therefore may not reflect its percutaneous kinetic processes before a systemic distribution equilibrium is reached. Temporal profiles of a topical penetration enhancer in the skin and in the body are important information for the development of dematologic preparations for the treatment of skin disorders.

KEY WORDS: skin penetration; penetration enhancer; hexamethylene lauramide; cutaneous blood; systemic disposition; skin flap.

INTRODUCTION

Percutaneous drug delivery for the treatment of topical or systemic disorders has received much attention. Limited percutaneous bioavailability of many compounds has been demonstrated (1,2), although it is theoretically possible to obtain complete absorption of topical doses. Compounds with penetration enhancement effects have therefore been utilized to promote skin penetration of drugs (3,4).

The penetration enhancement effects of hexamethylene lauramide (hexahydro-1-lauroyl-1H-azepine) have been evaluated using mouse skin both *in vitro* and *in vivo* (5). Ideally, an enhancer should exert its effect at the drug application site and then be readily eliminated by the body without adverse effects. Knowledge of the disposition kinetics of hexamethylene lauramide across the skin and in the body is therefore necessary to understand its action better.

The topical doses of many drugs from dermatologic formulations are invariably much smaller than systemic doses. The systemic drug concentrations after topical application are difficult to quantify because they usually fall below the assay detection limit. Additionally, the systemic disposition profile does not necessarily reflect drug profiles occurring in the local tissues, e.g., the sites of skin diseases. The ability to quantify drug concentrations in skin layers and in the circulating blood at the site of dose thus becomes critical in understanding the mode of action of topical drugs.

This study employed rats with isolated sandwich skin flaps which allowed the direct monitoring of drug concentrations in the cutaneous blood circulation at the dose site. Percutaneous and systemic temporal profiles of hexamethylene lauramide were investigated. Its effect on percutaneous absorption of hydrocortisone *in vivo* was evaluated using an experimental formulation.

EXPERIMENTAL

All solvents used in this study were high-performance liquid chromatographic (HPLC) grade. All other chemicals

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were reagent grade unless otherwise noted. Radioactive hexamethylene lauramide, [^{14}C-*carbonyl*]hexamethylene lauramide with a specific activity of 0.22 and 4.8 mCi/mmol, was custom synthesized by Wizard Laboratories. Tritiated hydrocortisone (sp act, 40 Ci/mmol) was obtained from New England Nuclear. All formulation vehicles contained 20% propylene glycol in ethanol.

Creation of Sandwich Skin Flaps

This procedure was modified from a previously reported method (6). Briefly, a piece of dermatomed skin (500 μm in thickness) was obtained from the dorsum of a fuzzy rat (250–350 g in weight) and then autografted to the epigastric pedicle flap on its ventral side. The epidermal side of the graft faced the abdominal musculature and a sandwich flap (a skin flap with split-thickness skin graft attached to its subcutaneous surface) was created. The abdominal wound was anatomically approximated by surgical staples. Scar tissues were allowed to develop around the wound to isolate the blood circulation of the flap from that of the surrounding area. After the graft grew to the pedicle flap (in about 2 weeks), the sandwich flap was raised and externalized from the body. Microvascular surgery was performed so that the flap was attached to the body by a stalk composed of the inferior epigastric vessels and nerve. These surgical procedures forced all the blood from the femoral artery to the sandwich flaps and then back to the femoral vein. There was minimal collateral circulation to the flap.

It usually required 2 weeks for the flaps to heal. These flaps had clinically normal-looking skin and similar extents of cutaneous blood flow as measured by laser Doppler velocimetry. Only healed flaps were used in experiments of percutaneous absorption.

Percutaneous Absorption Profiles of Hexamethylene Lauramide

Throughout the experiment, the rat was anesthetized by a bolus intramuscular injection of acepromazine and maintenance doses of a Rompun/ketamine mixture (1:1). A 100-μl dose (~20.5 μCi) of hexamethylene lauramide (~1.25%, w/w) was applied to a 4-cm^2 surface area on the host side of the skin flap.

Each anesthetized rat was restrained in a prone position throughout the 6-hr experiment. The femoral veins were exposed surgically to facilitate blood collection. Blood samples (50 μl) were collected at 30-min and at 60-min intervals from the ipsilateral epigastric vein and from the contralateral femoral vein, respectively.

At the end of the experiment, the rats were euthanized and the unabsorbed hexamethylene lauramide was removed from the skin flap with five alcohol swabs. A tape stripping technique (10 times) was used to remove the stratum corneum. The 4-cm^2 application site was removed from the graft side of the skin flap. The epidermis and dermis were then separated by gentle heating.

Quantitation of Urinary and Fecal Recoveries of Hexamethylene Lauramide After a Topical Dose

The urinary and fecal excretion profiles of total radioactivity following a topical application of hexamethylene lauramide to the host side of the skin flap were monitored. Six rats were used. Each rat was anesthetized for 6 hr postdose. The experimental vehicle contained 21% (w/w) ^{14}C-hexamethylene lauramide due to the low specific activity of the labeled material (0.22 mCi/mmol). A 100-μl dose was applied over 4 cm^2. Six hours postdose, the drug application site was swabbed five times with alcohol-soaked cotton swabs to remove any unabsorbed drug. Each rat was housed separately in metabolic cages for 6 days with free access to food and drinking water. Urine volume and fecal weights were recorded. Samples, urine and feces, were collected at 6, 24, 48, 72, 120, and 144 hr postdose.

Effect of Hexamethylene Lauramide on Skin Penetration of Hydrocortisone

Six rats received 100 μl of 1% ^{3}H-hydrocortisone (w/w) topically, while another four rats received a 100-μl dose containing 21% ^{14}C-hexamethylene lauramide and 1% ^{3}H-hydrocortisone. Rats were anesthetized throughout the experiment. Systemic and flap blood samples (50 μl) were collected every 30 min for 8 hr postdose. At the end of the experiment, five alcohol swabs were used to recover the unabsorbed dose. Tape stripping techniques were employed to recover drug in stratum corneum.

Analysis of Radioactivities

Radioactivities (dpm) in alcohol swabs, tape strips, metabolic cages, and urine were quantified by scintillation counting methods. The amounts of hexamethylene lauramide in the blood, epidermis, dermis, and feces were determined by scintillation counting (Beckman LS 1801 scintillation counter) following complete combustion of samples (Packard B306 tissue oxidizer).

The total radioactivity (% dose) collected in urine and/or feces was divided by the respective sampling interval to obtain the urinary or fecal excretion rate (% dose/hr). T_{mid} (hr) was the midpoint of each sampling interval. The elimination rate constant (k) was obtained by unweighted log-linear regression of the excretion rate $-T_{mid}$ data. The elimination half-life was (ln 2)/k.

Detection of Intact Hexamethylene Lauramide in Urine

An HPLC procedure was utilized to quantitate selectively the amount of intact hexamethylene lauramide in urine samples. The chromatographic system was composed of an automated injector (WISP, Waters, Milford, Mass.), a Beckman Model 114 pump (Berkeley, Calif.), a fatty acid analysis column (30 cm × 3.9 mm, Waters, Milford, Mass.), a variable-wavelength UV detector (Kratos SF 783, Ramsey, N.J.) set at 210 nm, and a Foxy fraction collector (ISCO, Inc., Lincoln, Neb.). The mobile phase (H_2O:CH_3OH:CH_3CN = 40:25:35, v/v; pH 3.5 by $HClO_4$) was delivered at 2 ml/min. The retention time of hexamethylene lauramide was 14 min. The HPLC effluent fractions were collected at 30-sec intervals. Radioactivity in each fraction was determined by liquid scintillation techniques.

RESULTS

After hexamethylene lauramide was topically applied to

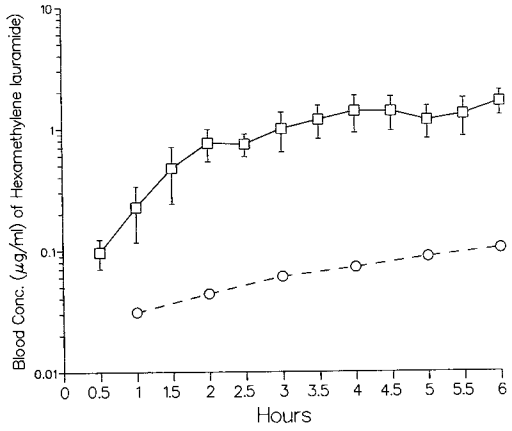

Fig. 1. The concentration of ^{14}C-hexamethylene lauramide in the flap blood (□) and in the systemic blood (○) during 6 hr after a topical dose to the host side of the sandwich skin flap in rats (mean ± SE; N = 6).

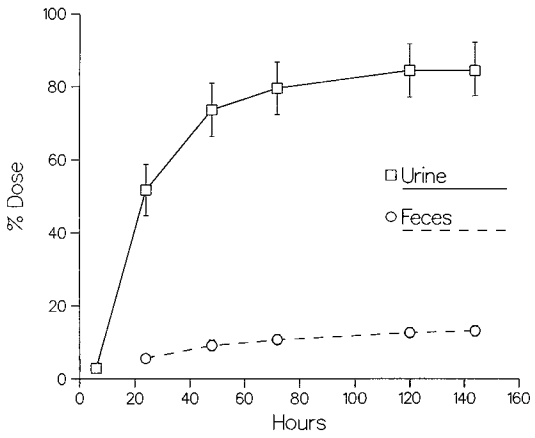

Fig. 3. The cumulative recoveries of ^{14}C-hexamethylene lauramide in urine and feces after a 6-hr topical application to the skin flap of rats (mean ± SE; N = 6).

the rat skin, it penetrated the stratum corneum, the viable epidermis, and some parts of the dermis before it appeared in the cutaneous capillary venules. The appearance of hexamethylene lauramide in the superficial inferior epigastric vein, into which the blood from the drug application site drained, was facile and detectable within 30 min postdose. Flap blood concentrations of hexamethylene lauramide increased slowly and steadily with time and reached relatively steady levels in 3 hr (Fig. 1).

The relatively steady levels in flap blood observed between 3 and 6 hr postdose indicated a steady state across the cutaneous tissues of the drug application site. The concentration gradient of hexamethylene lauramide across the skin layers at this steady state is shown in Fig. 2. The tissue weights of epidermis and dermis in this study were approximately 17 and 374 mg, respectively. The apparent concentration in epidermis was 19 times that in dermis, although both tissues contained similar amounts of drug, about 6–8% of the applied dose. The apparent hexamethylene lauramide concentration in the dermis was 163 times that in the cutaneous circulation, the flap blood. The concomitantly measured systemic blood concentrations steadily increased with time, indicating continuous entry of hexamethylene lauramide into the systemic circulation.

In the mass-balance study, about one-third of the hexamethylene lauramide dose remained unabsorbed in 6 hr. The absorbed dose was excreted in urine (85%) and feces (13%). The total dose applied was quantitatively recovered (97 ± 6%) as the unabsorbed dose (32 ± 6.3%) and as the drug that inadvertently adhered to the metabolic cage (1.3 ± 0.2%), in the urinary excreta (55.5 ± 5.1%), and in the feces (8.5 ± 0.7%). The cumulative recoveries of total radioactivity in urine and feces are shown in Fig. 3. The excretion rate of total radioactivity followed a log-linear fashion (Fig. 4). The terminal half-lives for urinary and fecal excretion were 17 and 30 hr, respectively. Only a trace amount of intact hexamethylene lauramide (less than 0.1% of the dose) was recovered in the urine.

The penetration of hydrocortisone across the rat skin is listed in Table I. Rats treated with hexamethylene lauramide had less unabsorbed hydrocortisone on the skin and more

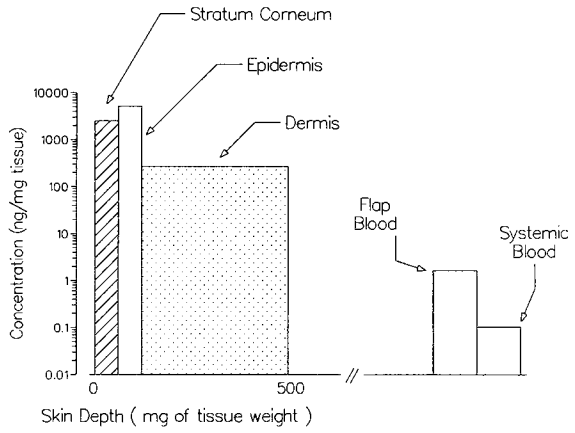

Fig. 2. The concentration gradient of ^{14}C-hexamethylene lauramide across the percutaneous tissues and the systemic blood at 6 hr after a topical dose to the skin flap of rats (mean ± SE; N = 6). The tissue weights for the stratum corneum, viable epidermis, and dermis were approximately 15, 17, and 374 mg, respectively.

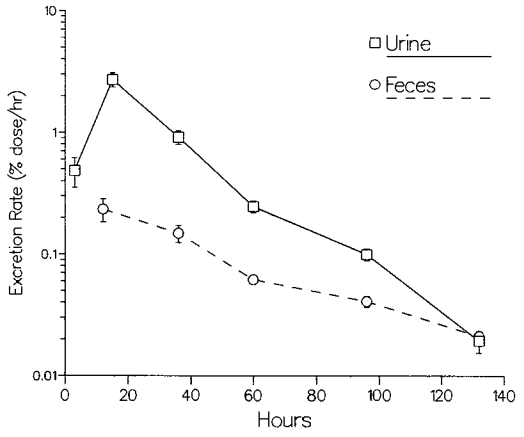

Fig. 4. The temporal profiles of excretion rate in urine and feces (mean ± SE; N = 6).

Table I. Percutaneous Distribution of ^{3}H-Hydrocortisone (1%, w/w) at 8 hr After a Topical Dose (Mean ± SE)

	Without hexamethylene lauramide	With hexamethylene lauramide
% dose unabsorbed*	58.5 ± 2.9	25.8 ± 7.4
% dose in stratum corneum*	1.47 ± 0.37	2.95 ± 0.97
Flap blood concentration (ng/ml)*	236 ± 130	806 ± 369
Systemic blood concentration (ng/ml)*	22 ± 8	76 ± 14
N	6	4

* $P < 0.05$ (two-tailed t test).

hydrocortisone in the stratum corneum. The average blood concentrations of hydrocortisone in the absence and in the presence of hexamethylene lauramide are also included in Table I. Figure 5 depicts representative profiles of ^{3}H-hydrocortisone in the flap blood and in the systemic circulation in the absence of and in the presence of hexamethylene lauramide. Hexamethylene lauramide enhanced the cutaneous blood concentrations of hydrocortisone by about 3.4-fold. The systemic blood concentrations of hydrocortisone, being much lower than the respective blood concentration at the drug application site, were also enhanced by similar proportions. The concentration–time profile of hexamethylene lauramide in the flap blood, simultaneously monitored using the dual-label technique, was similar to that when it was administered alone.

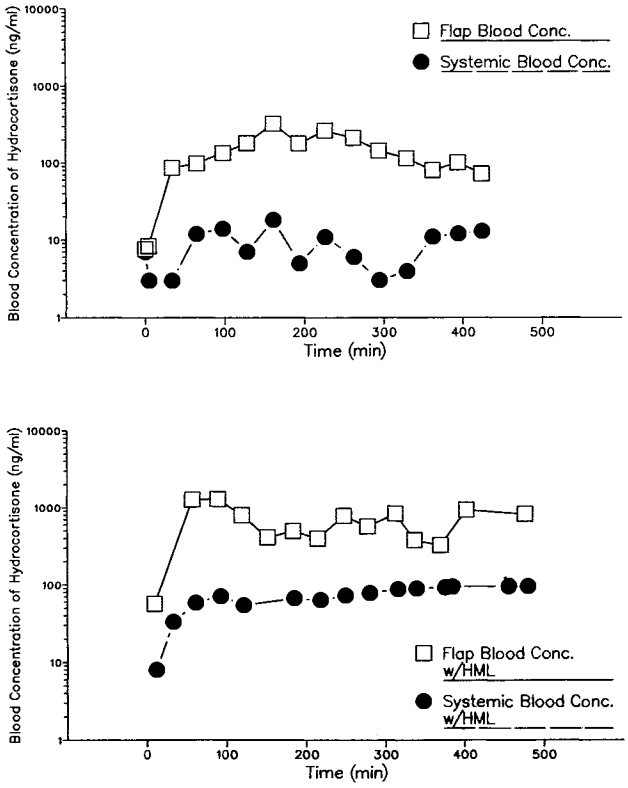

Fig. 5. Blood concentrations of ^{3}H-hydrocortisone in the absence (top) and in the presence (bottom) of hexamethylene lauramide.

DISCUSSION

In order to monitor directly the percutaneous absorption kinetics of drugs, rats with skin flaps were employed in this study. Each rat had an external sandwich skin flap attached to the body by the inferior epigastric vessels. Both sides of the skin flap had intact stratum corneum layers. Each flap had isolated blood circulation to allow us to monitor the drug concentration in the cutaneous bloodstream at the drug application site before it was available for systemic distribution. The systemic drug concentration was concomitantly measured via blood sampling at the contralateral femoral vein. Drug concentrations at the sites of topical action and in the systemic circulation can be differentiated using this model.

In 3 hr after a topical dose, hexamethylene lauramide reached a steady state at the cutaneous region of the site of application, despite its ascending systemic blood concentrations. The rising systemic blood concentrations demonstrated a continuous process of drug entry into the systemic circulation. If the study were carried out until after the absorption process ceased, hexamethylene lauramide in the skin layers would eventually reach a distribution equilibrium with the systemic circulation.

One topical dose of hexamethylene lauramide produced high tissue concentrations in the stratum corneum and the viable epidermis. The concentrations across the skin were as follows, in decreasing order: epidermis > dermis >> cutaneous blood. Assuming tissue densities of one, the apparent tissue concentrations of hexamethylene lauramide after the 1.25% topical dose were approximately 9, 18, 1, and 0.006 mM in the stratum corneum, viable epidermis, dermis, and cutaneous blood, respectively. The large difference between the drug concentrations in the skin and those in the local circulation was due to the concentration of the lipophilic drug in the lipoidal regions of extravascular tissues, especially in the stratum corneum, prior to the drug's diffusive movement into the local circulation. Relative to its concentrations in the flap blood, the systemic hexamethylene lauramide concentrations were low due to the processes of tissue distribution, drug metabolism, and drug excretion.

About 13% of the percutaneously absorbed hexamethylene lauramide was recovered in feces. This suggested biliary excretion of ^{14}C-hexamethylene lauramide-derived species or direct diffusion of these species from systemic circulation into the gastrointestinal contents. Hexamethylene lauramide was extensively metabolized to polar species as indicated by the HPLC analysis. No identification of the metabolites was attempted.

More hydrocortisone was percutaneously absorbed when hexamethylene lauramide was topically coadministered than when only hydrocortisone was applied. Although the hexamethylene lauramide concentration in the formulation was higher than desired, the penetration enhancement effect on hydrocortisone was still observed. This was reflected by the decreased hydrocortisone remaining on the skin surface, elevated drug content in the stratum corneum, and increased cutaneous and systemic drug concentrations in the blood.

The significance of this study is manifold. The percutaneous absorption of hexamethylene lauramide was rapid. The apparent concentrations of hexamethylene lauramide

varied greatly between the epidermis and the cutaneous blood circulation. The results demonstrated that hexamethylene lauramide concentrated in the stratum corneum and the viable epidermis, presumably in the lipoidal regions between cells. This was consistent with its mechanism as a skin penetration enhancer. The study also demonstrated, for the first time, that the steady-state concentration gradient of a topically applied drug was established prior to its systemic distribution equilibrium. The skin-flap model enabled us to monitor simultaneously the local and systemic levels of a penetration enhancer and to understand the time course of the enhancement action. Both percutaneous absorption and retention of drug in the skin layers should be optimized for successful treatment of skin disorders. The temporal profile of topical penetration enhancers is therefore an important selection criterion for the development of dermatologic drug preparations.

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