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In-vitro Skin Pharmacokinetics of Acitretin: Percutaneous Absorption Studies in Intact and Modified Skin from Three Different Species using Different Receptor Solutions

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Abstract—The aromatic synthetic retinoid acid derivative, acitretin, is efficacious in several cutaneous diseases. Its toxicological profile makes a topical form with no or reduced systemic adverse effects desirable. Direct application of a topical acitretin formulation might result in therapeutic skin concentrations at the site of the disease while minimizing systemic exposure. The present studies define the percutaneous absorption characteristics of acitretin from an isopropylmyristate formulation. We investigated, in-vitro, (1) the role of receptor solution variations, (2) the role of skin modifications, (3) the influence of skin from three different species on the absorption of topically applied acitretin and (4) the drug distribution within the skin. Addition of solubilizers (Polyethylenglycol-20 and albumin) to the receptor solutions improved the flux of acitretin through monkey skin, whereas the acitretin concentration in the skin was not affected by the various receptor solutions used. Acitretin flux through tape-stripped monkey skin and dermis was only slightly higher than through intact skin. Acitretin concentration in human skin was significantly higher than in rhesus monkey or guinea-pig skin. Topical application of acitretin can produce dermal concentrations in excess of those achieved by therapeutic oral doses.

The synthetic retinoic acid derivatives represent an important tool in modern therapy for various dermatologic diseases. The aromatic compounds etretinate (Ott & Bollag 1975) and acitretin (Hänni 1978; Paravicini et al 1981) (Fig. 1), the active metabolite of etretinate, are efficacious in several cutaneous diseases (Binazzi & Cicilioni 1979; Viglioglia 1980; Christenesen et al 1981; Geiger et al 1984; Schuppli 1985). Both compounds are only available in an oral dosage form. Their toxicological profile includes teratogenicity, mucosal disturbances and other hypervitaminosis A related effects (Chen 1985; Cunningham 1985; Melnik et al 1987). Etretinate with a terminal elimination half-life of up to 120 days is metabolized by hydrolysis to acitretin, the corresponding free aromatic acid, which exhibits a considerably shorter half-life of 2 to 4 days (Paravicini et al 1981; Vahlquist et al 1988: Brindley 1988). Despite the pharmacokinetic advantage of acitretin, being rapidly eliminated, the compound shares the toxicological profile of etretinate. These adverse effects make a topical form of acitretin with no or reduced systemic side effects desirable. Certain factors suggest that the flux from a topical formulation may be limited. Among them are low solubility in most vehicles and photolability (Halley & Nelson 1979; Lehman et al 1988). Even though its solubility may limit incorporation of the compound into a vehicle, it is proposed that direct application of a topical acitretin formulation to the skin might result in therapeutic skin concentrations (Rollmann & Vahlquist 1983; Laugier et al 1989) at the disease site while minimizing systemic exposure. The present studies investigate the percutaneous absorption characteristics of acitretin from an isopropylmyristate formulation and evaluate the feasibility of its topical administration.

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Materials and Methods

Chemicals

Acitretin (a gift from Hoffmann-La Roche Inc., Nutley, NJ, USA), labelled at position 7 with ^{14}C had a specific activity of 56 mCi $mmol^{-1}$. The compound was protected from light and stored at −30°C. Radiochemical purity was greater than 98%. Isopropylmyristate (IPM) and bovine serum albumin were obtained from Sigma, St Louis, MO, USA. Phosphate buffered saline (pH 7·3–7·4) (PBS) was from the cell culture facility, University of California, San Francisco, USA and the cell culture medium, RPMI 1640 with L-glutamine, from Gibco (Grand Island, NY, USA). Skin solubilizer, Soluene-350, was purchased from Packard Instrument Co., Inc. (Doves Grove, IL, USA), scintillation fluid, Ready Value,

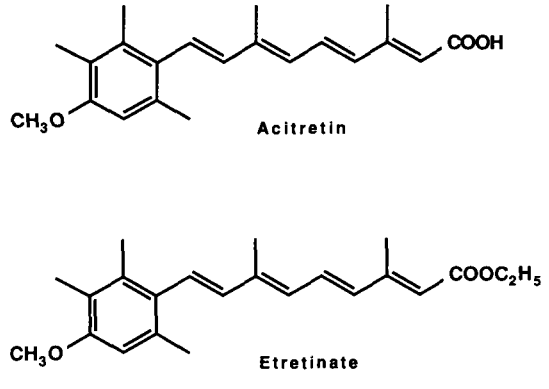

FIG. 1. Chemical structures of acitretin and etretinate.

was from Beckmann Instruments (Fullerton, CA, USA) and Polyethylenglycol-20 (Volpo 20; Lot # 8084) (PEG-20) was a gift from Croda Inc. (New York, NY, USA).

Experimental protocol

Acitretin concentrations were determined in skin compartments such as dermis, epidermis and stratum corneum from guinea-pig, monkey and man, to investigate in-vitro (1) the role of receptor solution variations, (2) the role of skin modifications, (3) the influence of skin from three different species on the absorption of topically applied acitretin and (4) the drug distribution within the skin.

Preparations

Hairless guinea-pig skin (GP-S). Skin from the back was obtained from female hairless guinea-pigs, 450 g (Charles River Inc. Wilmington, MA, USA). Subcutaneous fat and a portion of the reticular dermis was removed by scraping and slicing with a #2 scalpel blade (Bard-Parker; Rutherford, NJ, USA). The skin was used immediately.

Rhesus monkey skin (RhM-S). Abdominal skin was taken from a rhesus monkey (female, 8 years) and prepared as above. Hair was closely shaved avoiding abrasion. The skin was either stored at 4°C in PBS for a maximum of three days or used immediately.

Human skin (H-S). Human skin from the thighs was obtained at autopsy from a 54 year old male Caucasion within 24 h of death. Skin free of visual defects and dermatological disorders was obtained with a dermatome (Padgett, Mod. B; Kansas City, MO, USA). The skin was either stored at 4°C in PBS for a maximum of 3 days or used immediately.

General design. Flow-through diffusion cells (Gummer et al 1987) (Berkeley Glass Apparatus, Inc. LG-1084-SPC, Berkeley, CA, USA) were used. The cells allowed 5 cm^2 of the GP-S and the RhM-S or 1 cm^2 of the H-S portion to be exposed to ambient temperature (22°C) air, and humidity while the dermis was bathed at 37°C in 3 and 4 mL receptor solution, respectively. The receptor solution was stirred with a teflon coated magnetic bar at 600 rev min^{-1}. The receptor solution was collected hourly (5 mL) for 24 h, using an automated fraction collector (Retriever IV, Isco, Inc., Lincoln, NJ, USA). The receptor solution was either pure PBS or PBS with 3% PEG-20, or pure CCM, or CCM with 3% albumin. PEG-20 and albumin were used to increase the solubility of acitretin in the receptor solution. The skin was modified by repeatedly (15 times) applying and removing cellophane tapes (Scotch 600, 3M, St Paul, MN) from the isolated skin. Dermis was prepared from isolated skin by heat separation on a slide warmer at 50°C for 50 s (Surber et al 1990). RhM-S was used to investigate the influence of the various receptor solutions and the influence of tape-stripping. For the skin modification experiments and the species variability experiments PBS with 3% PEG-20 was used. All experiments were performed on at least six replicates (n = 6). A standardized procedure was used to prepare and apply the acitretin formulation. Acitretin (100 mg) in 5·0 mL IPM was stirred for 24 h at 22°C. The suspension was centrifuged (22°C, 5300 *g*, 15 min). The supernatant (3·5 mL) was mixed with [^{14}C]acitretin and equilibrated for 24 h at 32°C. The standardized procedure for formulation preparation produced a saturated solution of 330 μg acitretin mL^{-1} IPM yielding maximum thermodynamic activity. Acitretin formulation (500 μL) was pipetted onto the surface of the excised skin in the cell. Due to the photolability of acitretin all experimental procedures were performed under total light protection. A standardized procedure was applied after 24 h to remove remaining drug formulation from the skin and to prepare the various skin samples for analysis. The skin site was wiped with cotton balls soaked with liquid soap-water solution (1 : 5) (liquid ivory; Procter & Gamble, Cincinatti, OH, USA) and then with water, followed by the liquid soap-water solution again and then once with water and once with a dry cotton ball to remove residual soap and water. The whole skin was removed from the cell. Exposed skin was separated from unexposed skin and both specimens weighed. The dermal–epidermal junction loosens during the 24 h exposure to the receptor solution and stratum corneum/epidermis could easily be separated from the dermis by lifting with a scalpel. Both stratum corneum/epidermis and dermis were solubilized in Soluene-350. In order to apply mass balance (Bucks et al 1988) the unexposed skin was also solubilized, instruments and cell assemblies were washed with an acetone–methanol solution (1 : 2). In all experiments more than 95% of the applied dose was recovered.

Sample handling. Acitretin was quantitated radiometrically on a scintillation counter (Tri-Carb 4640, Packard Instrument Co., Inc., Dowers Grove, IL, USA). Acitretin was calculated from the amount of radioactivity measured in the specimens. The entire receptor solution (5 mL) and the wash solution (cotton balls) were mixed with 15 mL Ready Value for scintillation counting; 1 mL of the digested skin samples and 1 mL of the methanol–acetone wash solution were mixed with 5 mL Ready Value. To avoid chemiluminescence samples remained in complete darkness at 5°C for 1 week until counting.

Results

Variation of receptor solution

The lowest acitretin permeation was seen with the receptor solutions without additives (Fig. 2). Addition of 3% PEG-20 or 3% albumin produced a 5- to 7-fold increase of permeation. No significant differences in permeation were revealed either between the PBS and the CCM receptor solutions or between the PBS and the CCM receptor solutions with the additives ($P \leq 0{\cdot}05$). Steady state flux was not achieved in any of the experiments within 24 h. The dermis and the stratum corneum/epidermis compartments showed similar acitretin concentrations in all four experiments and ranged from 0·3 to 0·4 μg g^{-1} hydrated tissue and 5·0 to 7·8 μg g^{-1} hydrated tissue, respectively (Table 1). The use of different receptor solutions did not significantly influence the acitretin concentration in the dermis or in the stratum corneum compartment.

Skin modification

The lowest acitretin permeation was seen in intact skin (Fig. 3).

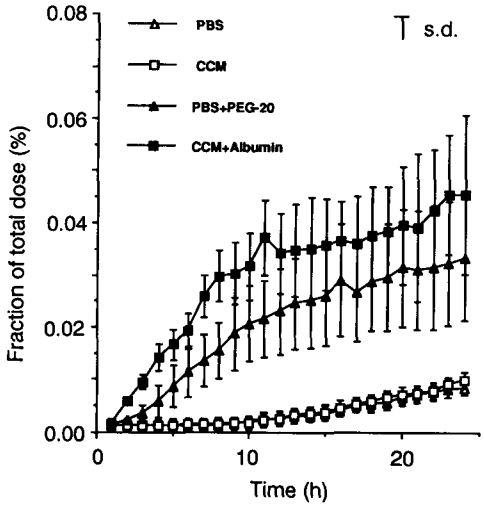

FIG. 2. Time course of acitretin permeation through rhesus monkey skin using four different receptor solutions. PBS, phosphate buffered saline. CCM, cell culture medium. PEG-20, Polyethylenglycol-20.

Table 1. In-vitro penetration of acitretin into rhesus monkey skin using three different receptor solutions (calculated as μg acitretin (g hydrated tissue)$^{-1}$).

	Stratum corneum/epidermis	Dermis
PBS	5·8±2·1	0·3±0·1
CCM	6·5±2·3	0·4±0·2
PBS+3% PEG−20	7·8±2·0	0·3±0·1
CCM+3% albumin	5·0±1·8	0·4±0·1

PBS, phosphate buffered saline, CCM, cell culture medium.

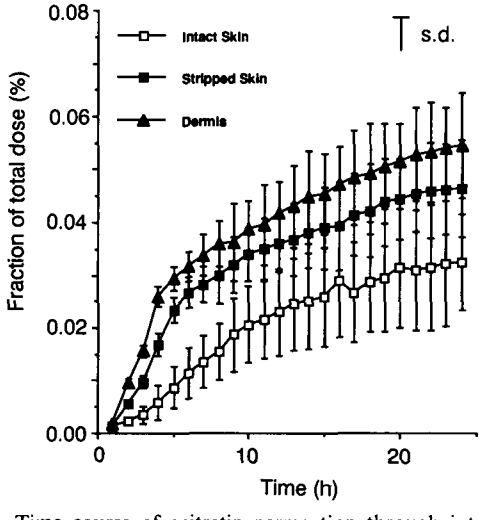

FIG. 3. Time course of acitretin permeation through intact and modified rhesus monkey skin.

Permeation through stripped skin and dermis was higher than in intact skin; a significant difference was revealed between intact skin and dermis ($P \leq 0{\cdot}05$). Steady state flux was not achieved in any of the experiments within 24 h. The compartmentalization of acitretin is shown in Table 2. The dermis compartments in intact and stripped skin showed similar acitretin concentrations. In the heat separated dermis the acitretin concentration was more than 2-fold higher.

Species variation

The influence of the species variation on acitretin absorption is summarized in Table 3. Comparing acitretin penetration, drug concentration in human dermis was significantly higher than in rhesus monkey dermis or guinea-pig dermis ($P \leq 0{\cdot}05$). Permeation through intact guinea-pig skin was significantly higher than in rhesus monkey skin and human skin ($P \leq 0{\cdot}05$).

Discussion

Variation of receptor solution

The receptor solution has an important influence on the in-vitro absorption of compounds with low solubility (Bronaugh 1989). The drug's low solubility may govern its transfer from the skin to the receptor solution and therefore can become the rate-limiting step in percutaneous absorption. Solubilizers are commonly used to increase the solubility of the penetrant in the receptor solution to assure sink conditions (Bronaugh & Stewart 1984; Bronaugh 1989). Despite reports that up to 6% PEG-20 in the receptor solution does not affect the absorption characteristics of the skin (Bronaugh 1989), PEG-20 and additionally CCM with and without albumin was utilized to mimic a biologically more realistic environment for the skin (Holland et al 1984; Kao et al 1985; Klain et al 1985). The addition of PEG-20 and albumin to the receptor solutions highly influenced acitretin permeation (Fig. 2). The receptor solutions without additives allowed only minimal permeation, suggesting that insufficient drug transfer from the skin into the receptor solution may have limited permeation. The compartmentalization showed that the acitretin concentration in the dermis compartment was unaffected by the receptor solutions (Table 1). In all instances, the acitretin solubility in the 4 receptor solutions was sufficient (< 10% of the saturation solubility) to maintain a favourable driving force and to assure reasonable collection of permeant (Skelly et al 1987). It may be suggested that saturation of dermis with acitretin occurred due to saturation of binding sites in the skin and saturation of the inter- and intracellular liquid (Gibaldi & McNamara 1978; Bickel 1985; Lehman et al 1988).

Skin modification

Percutaneous absorption is usually determined with intact skin. An estimate of absorption for diseased skin is determined by inflicting some trauma to the skin as a model system. This extends from the assumption that diseased skin is damaged skin. The assumption then follows that percutaneous absorption through diseased or damaged skin is enhanced, and that the skin's ability to protect against intrusion by a chemical is impaired. Among various physical procedures cellophane tape-stripping is used to disrupt the barrier to percutaneous absorption. Acitretin permeation through tape-stripped skin and heat separated dermis were higher than in intact skin (Fig. 3). No significant difference was revealed between permeation through intact skin and stripped skin. This leads to the presumption that stratum corneum may not be the rate-limiting step (barrier) in

Table 2. In-vitro penetration of acitretin into intact and modified rhesus monkey skin (calculated as μg acitretin (g wet tissue)$^{-1}$).

	Stratum corneum/epidermis	Epidermis	Dermis
Intact skin	7·8±2·1	n.a.	0·3±0·1
Tape-stripped skin	n.a.	6·9±2·9	0·3±0·1
Dermis (heat separated)	n.a.	n.a.	0·7±0·5

n.a. not applicable.

Table 3. In-vitro percutaneous absorption of acitretin through skin of three different species using PBS with 3% PEG-20 as the receptor solution.

	Guinea-pig	Rhesus monkey	Man
Penetration*			
Stratum corneum/epidermis	3·3±1·2	7·8±2·1	11·3±4·5
Dermis	0·8±0·3	0·3±0·1	3·0±0·6
Permeation Δ	4·6±0·6	0·8±0·3	1·3±0·3

* Calculated as μg acitretin (g wet tissue)$^{-1}$. Δ Calculated as μg cm^{-2} acitretin permeated through skin within 24 h.

percutaneous absorption of acitretin. Early in-vivo studies with hydrocortisone in man showed that tape-stripping of the skin did not entirely remove skin barrier properties (Feldmann & Maibach 1965). The compartmentalization of acitretin showed similar dermis concentrations in intact and stripped skin (Table 2). As with the variation of receptor solution, it may be suggested that saturation of dermis with acitretin occurred due to saturation of binding sites in the skin and saturation of the inter- and intracellular liquid (Gibaldi & McNamara 1978; Bickel 1985; Lehman et al 1988). Although we applied a standardized procedure to decontaminate the skin by an efficient wash procedure, complete decontamination of the skin layer in direct contact with the formulation cannot be guaranteed. This may explain the higher acitretin concentration in heat-separated dermis.

Species variation

We wished to study percutaneous absorption in human skin. However, human skin is not readily available and therefore comparative information on percutaneous absorption from animal skin is desirable. Because of a slightly different skin preparation technique in humans and animals the permeation data was not interpreted (Table 3); human skin was prepared with a dermatome at a thickness of 0·5 mm, whereas animal skin was prepared by manual removal of subcutaneous fat and portions of reticular dermis by scraping and slicing with a scalpel revealing a skin membrane of about 0·8 mm thickness. Comparing the dermis compartments, acitretin concentrations were considerably higher in human dermis than in monkey or guinea-pig dermis. Dermal acitretin concentration in human skin resembled the findings of Lehman et al (1988) using a similar experimental approach. Our experiments were performed three times with sets of six cells revealing the same results. It may be suggested that acitretin concentration in human dermis is related to a distinct affinity of acitretin to human tissue (Surber et al 1990).

In our in-vitro experiments topical application of acitretin resulted in much higher drug concentrations within the dermis than observed following oral administration of etretinate (Rollmann & Vahlquist 1983;) or acitretin (Laugier et al 1989) in man. In human skin a single topical dose delivered in-vitro a dermal acitretin concentration of ~3000 ng (g hydrated tissue)$^{-1}$ (Table 3), exceeding that achieved by oral administration of etretinate or acitretin by 30-fold (Rollman & Vahlquist 1983; Laugier et al 1989).

Even though the experimental conditions met the standard requirements (Skelley et al 1987) for in-vitro percutaneous penetration studies, it is noteworthy that acitretin concentration in the skin remained unaffected by differences in the receptor solution or by skin modification. The data from the species variation experiments may indicate that acitretin affinity to tissue is species dependent. It is now necessary to explore whether the observations made with acitretin apply to other compounds. The observations reported here were obtained from controlled in-vitro experiments. How well this data reflects in-vivo skin pharmacokinetics of acitretin should be determined.

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