

In Vivo Skin Penetration of Acitretin in Volunteers Using Three Sampling Techniques

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Etretinate and acitretin are given orally to treat psoriasis and various keratinization disorders. Acitretin, the main active metabolite of etretinate, has the pharmacokinetic advantage of being rapidly eliminated, but it shares etretinate's toxicologic profile. Thus a topical delivery of acitretin with no or reduced systemic adverse effects is desirable. To characterize the therapeutic potential of topically delivered acitretin, we quantitatively assessed its percutaneous penetration in healthy human volunteers. Additionally, three skin sampling techniques, the punch biopsy, the shave biopsy, and the suction blister technique, were validated to quantitate acitretin in the skin. The results suggest that topical delivery of acitretin renders skin concentrations which exceed those reported after oral administration of etretinate or acitretin. However, because of possible interlaminar drug contamination, drug localization within a particular skin compartment cannot be determined.

KEY WORDS: acitretin; human; topical; percutaneous penetration; skin sampling techniques.

INTRODUCTION

The development of retinoids and their clinical applications have revolutionized therapeutic management in the field of dermatology. Despite recent advances, serious adverse effects such as teratogenicity (1), bone toxicity (2), and lipid metabolism disorders (3) render it necessary to continue the search for safer or more effective retinoids. Synthetic retinoids should ideally exert therapeutic activity without accumulating in the organism and without causing serious adverse effects. The oral administration of the two aromatic compounds etretinate (4) and acitretin (5) (Fig. 1) has made it possible to treat psoriasis and various keratinization disorders. Acitretin is the hydrolysis product of the ethyl ester etretinate and thus the main metabolite of etretinate (6). The antipsoriatic activity of acitretin has been demonstrated (7). The terminal half-life of etretinate is up to 3 months (8,9). Due to the potential teratogenic effect of the drug, the clinical applicability is therefore reduced. For acitretin a considerably shorter terminal half-life of up to 4 days has been reported (10). Despite this pharmacokinetic advantage of being eliminated much faster, acitretin has the same spectrum of adverse effects as etretinate.

Assuming that the skin is the target organ of acitretin

and the therapeutic response is a function of drug concentration in the skin, topical delivery of acitretin with no or reduced systemic adverse effects could be effective and safe for the presently approved indications. Despite some unfavorable physicochemical properties of acitretin such as low solubility in an acceptable vehicle and photolability, promising results from previous studies with the present and similar formulations (11–13) led us to focus our attention on the skin uptake of acitretin after topical administration in humans.

The present study addresses the question whether the topical administration of acitretin can produce in humans a drug concentration in the skin which is comparable to the acitretin concentration that is reported to be clinically effective after oral administration of acitretin or etretinate. Additionally, three skin sampling techniques, the punch biopsy, the shave biopsy, and the suction blister technique, were validated to quantitate acitretin in the skin.

MATERIALS AND METHODS

Test Formulation and Chemicals. Acitretin formulations were prepared by Hoffmann La Roche Inc. (Nutley, NJ). The formulation contained 330 µg acitretin/mL isopropylmyristate including 2.22 µCi [7-¹⁴C]acitretin. Chemical and radiochemical purity and formulation homogeneity were monitored throughout the study by thin-layer chromatography with a RTLC scanner (Radiometric Instruments and Chemical Co., Tampa, FL). Radiochemical purity was greater than 98.5%. Tissue solubilizer, Soluene-350, was purchased from Packard Instrument Inc. (Downers Grove, IL), scintillation counter cocktail (SCC), Ready Value, was from Beckmann Instruments (Fullerton, CA), dimethyl sulfoxide (DMSO) was from Sigma Inc. (St. Louis, MO), and the carbon-14 SCC for ¹⁴CO₂ counting was from Harvey Instruments Corp. (Hillsdale, NJ).

Human Volunteers. Nine healthy volunteers (male; mean age, 30.3 years; mean weight, 71.4 kg) took part in the study. A medical history was elicited from each and all underwent a complete physical and laboratory examination. All volunteers were free of any preexisting dermatologic disease, had normal blood values, and had not been previously treated with any natural or synthetic retinoids. After briefing, they were asked to sign a consent form. The trial was approved by the local ethics committee.

Dosing. Three occlusive application devices were placed separately on the upper outer quadrants of the volunteer's buttocks. The application devices were constructed of a polypropylene chamber (Hill Top; Hill Top Research, Miamitown, OH), modified with a dark tape covering to prevent the passage of light. Each application device (2.5 cm²) contained 450 µL acitretin formulation, yielding a single dose of 148.5 µg acitretin per device and a total dose of 445.5 µg acitretin per subject. Dosing and skin sampling were performed under minimal light exposure (e.g., yellow light, total light protection).

General Design. After a period of 24 hr, each application device was removed and placed separately in 100 mL DMSO. After a 7-day equilibration period 5 mL of the DMSO solution was mixed with SCC. Each application site

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Acitretin: Ro 10-1670

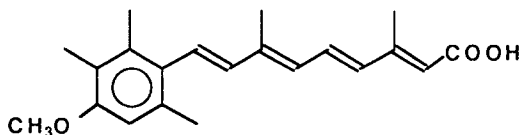


Fig. 1. Retinoid structure.

underwent a series of washings with wet, soapy, and dry cotton balls in order to collect any remaining compound on the skin surface ("mass balance" technique) (14). Next, the application area was covered with a plastic template exposing only the zone (2.5 cm²) previously exposed to the formulation. The sites where a punch and a shave biopsy were to be performed were cellophane tape-stripped (Scotch 600; 3M, St. Paul, MN) to glistening to collect approximately all stratum corneum (15 times). At the site where a suction blister was to be raised, only one tape strip was taken. To minimize the chance of infection, each site was then swabbed with an isopropyl alcohol pad. Each cotton ball, each tape, and the pads were placed separately in vials and SCC was added. After disinfecting one biopsy was performed at each of the three sites, including (i) a punch biopsy, (ii) a shave biopsy, and (iii) a suction blister. (i) The application site was anesthetized by injecting 0.5 mL of 2% lidocaine with 1:100,000 epinephrine subcutaneously. A 4-mm punch biopsy was taken from the center of the application site. The entire skin specimen consisting of epidermis, dermis, and some subcutaneous tissue was weighed (= > mg wet tissue), then solubilized in 1 mL Soluene-350 in a vial. After solubilization SCC was added. (ii) The application site was anesthetized as described above. A thin (0.5-mm) stainless-steel template with a 7-mm hole was pressed firmly to the site so that the underlying tissue popped through the hole. The tissue was removed with a lateral sawing motion of a halved super stainless steel blade, G4 (Gillette). The skin specimen consisting of epidermis and some dermis was weighed (= > mg wet tissue), then treated as described above. (iii) A suction blister apparatus (Dermovac; Medko Medical, Espoo, Finland) was used to form the blisters. The suction cup was applied to the center of the application site and a pressure of 40 Pa was sustained for ~2 hr to create three blisters with a diameter of 4 mm. Once the blister was formed, the suction blister fluid was aspirated into a preweighed syringe and was then weighed again (= > mg blister fluid). The suction blister fluid was placed in a vial and SCC was added. The three blister skin specimens were removed using a scalpel blade, then solubilized in 0.5 mL Soluene-350 in a vial. After solubilization SCC was added. In eight of nine subjects both feces and urine were collected for a period of 14 days starting the day of drug application. A 1-mL urine sample was collected from each subject prior to the placement of the acitretin devices to act as a blank for background radiation. Twenty to forty urine and feces samples from each day and each volunteer were analyzed for radiolabeled ¹⁴CO₂ after oxidation. Acitretin and metabolites were quantitated radiometrically by scintillation counting (Tri-Carb 4600; Packard Instrument Inc., Downers Grove, IL). Amounts of acitretin and metabolites were calculated from the amount of radioactivity measured in the specimens and are expressed as ng-eq/g wet

tissue. Prior to counting all samples were stored at 5°C in the dark for 1 week to allow chemiluminescence to subside. Oxidation of urine and fecal samples was carried out with an oxidizer (X-500; Harvey Instrument Corp., Hillsdale, NJ).

RESULTS

The "mass balance" technique (14) established an overall recovery of more than 92% of the applied dose. During the 14-day collection period acitretin could not be detected in the urine or in the feces.

Table I and Fig. 2 show acitretin and metabolites concentrations calculated from the radioactivity measured in the specimens and the various skin compartments to which the acitretin and metabolites concentrations are referred. The punch biopsy includes epidermis, dermis, and subcutaneous tissue. The shave biopsy is referred to as "epidermis," although it contains small amounts of papillary dermis. The blister skin specimen includes stratum corneum and epidermis. The blister fluid is representative of the interstitial fluid. The highest accumulation of acitretin and metabolites was seen in the stratum corneum/epidermis compartment of the BS. Less pronounced was the acitretin and metabolites concentration in the "epidermis" from the shave biopsy and in the skin specimen from the punch biopsy. In the blister fluid, only a marginal amount of acitretin and metabolites was detected.

DISCUSSION

We addressed the question whether the topical administration of acitretin can produce in humans a skin concentration which is comparable to the acitretin concentration that is reported to be clinically effective after oral administration of acitretin or etretinate. Three skin sampling techniques, commonly utilized in dermatology, were evaluated to quantitate acitretin in the skin.

After removal of the stratum corneum by tape stripping, the cylinder-shaped punch biopsy consists of epidermis, dermis, and some subcutaneous tissue weighing 34.5 ± 10.7 mg. The three compartments can be easily separated (12,15). However, the separation procedures involve a series of manipulations which may affect the drug concentration in the skin, e.g., cross-contamination of another skin compartment. Furthermore, detection of low drug concentrations in the entire skin specimen may be prevented by the further separation of the skin specimen. Therefore the entire biopsy was dissolved. The superficial shave biopsy is referred to as "epidermis," although it contains small amounts of papillary dermis. For an experienced operator it is possible to adjust the cutting depth so that the skin sample will be uniformly

Table I. Acitretin and Metabolite Concentration (ng-eq/g Wet Tissue)

Specimen			
Punch biopsy	Shave biopsy	Blister skin (BS)	Blister fluid (BF)
155 ± 150	358 ± 160	5370 ± 182	86 ± 60

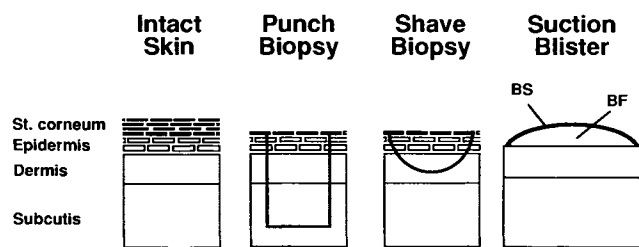


Fig. 2. Schematic diagram of intact skin (left) and the skin specimens taken using three sampling techniques. BS, blister skin; BF, blister fluid.

composed of about 80% epidermis (16). Using the metal template technique a uniform skin sampling was assured yielding ~7-mm-diameter epidermis specimens of 16.6 ± 4.6 mg. Acitretin and metabolites concentrations of 155 ± 150 eq-ng/g tissue in the punch biopsy and 358 ± 160 eq-ng/g tissue in the shave biopsy were determined. Despite the considerable variations, one may presume from these data that the drug is mainly located in the epidermis and upper dermis rather than in the deeper dermis and subcutaneous tissue.

The development of well-defined suction blisters has been used extensively to investigate the pharmacokinetics of systemically administered drugs acting in the skin (17,18). The suction blister approach has erratically been used to determine drug concentrations in the skin after topical administration (19). From the skin sampling methods used in this study, the suction blister technique is the least invasive method to collect tissue and tissue fluid. From the three suction blisters a total of 70.9 ± 23.6 mg blister fluid was harvested. The fluid is referred to as "interstitial fluid" (20). The blister skin (total area, 0.38 cm^2), referred to as "stratum corneum/epidermis," is difficult to weigh accurately. Therefore the weight was calculated assuming that the specific gravity of the blister skin is 1.32 mg/mm^3 (21) and the skin thickness is 0.02 mm. This presumption results in a ~1-mg blister skin specimen per volunteer. Our own experimentally determined stratum corneum/epidermis sheet weights from cadavers were $3.7 \pm 0.5 \text{ mg/100 mm}^2$ (15). This results in a ~1.4-mg skin specimen per volunteer. As one would expect, the highly lipophilic acitretin accumulated in the stratum corneum/epidermis compartment. Based on the calculated weight the stratum corneum/epidermis contains ~5370 ng-eq acitretin and metabolites/g wet tissue, and from the experimentally determined weight the stratum corneum/epidermis contains ~3800 ng-eq/g wet tissue. Only marginal drug concentrations could be detected in the blister fluid. In the case of systemic dosing the drug migrates from a dense capillary network to the skin. After equilibrium, drug concentrations in the blister fluid are indicative of drug concentrations in the blood. After topical administration the drug migrates from the stratum corneum/epidermis into the suction blister fluid, whence it is then removed by the capillary network located directly below the dermoepidermal junction. In this case the capillary network represents a perfect "sink" for the drug, possibly resulting in a low drug concentration in the blister fluid.

Rollman *et al.* (9) determined etretinate concentrations in shave biopsies of 27 patients after oral retinoid administration. Their methodology required the hydrolytic conver-

sion of etretinate to the acid form, acitretin. The reported concentrations were those of both species, etretinate and acitretin. They showed that dermal and epidermal retinoid concentration after 4 weeks of therapy (20–50 mg etretinate/day) achieved a level of ~110 ng/g tissue. Laugier *et al.* (12) dosed four patients with severe psoriasis with 30 mg/day acitretin for 1 month. In biopsies of involved and uninvolved skin 185 ng acitretin/g and 96 ng acitretin/g tissue, respectively, were detected. In our experiments the topical application of acitretin resulted in a concentration within the epidermis slightly higher than those observed following oral administration of etretinate and acitretin. A single topical dose of acitretin delivered an epidermal concentration of ~360 eq-ng/g tissue. This concentration is comparable to the acitretin concentration which is achieved by oral administration of etretinate or acitretin. Assuming now that the epidermis is the target organ for acitretin and the therapeutic response is a function of dermal or epidermal concentration, topical delivery of acitretin should, in theory, be effective.

All three skin sampling techniques have been used by various investigators to quantitate drugs or xenobiotics in the skin. Each technique gives access to distinctive skin compartments. The information gained is a *rough* estimate of the chemical distribution within the skin. Because of possible interlaminar drug contamination, accurate and specific information on drug localization within a particular skin compartment following both routes of administration is *not* obtained by the methods used. One may postulate that drug concentration at a particular site within the skin following both routes of administration could be different because of the direction of the drug concentration gradient. In a comparison of experimentally determined epidermal drug concentrations following oral and topical administration of aciclovir using comparable methodologies, Parry *et al.* (22) showed that topical administration of aciclovir produced significantly greater drug concentrations in the *entire* epidermis than oral administration. However, their theoretical prediction for drug concentration in the *basal* epidermis demonstrated that aciclovir concentration in the *basal* epidermis was greater following oral administration than with topical administration. The lack of clinical efficacy with topical aciclovir supports the theoretical prediction that only the oral administration delivers an adequate amount drug to the target site within the skin.

In summary, conventional skin sampling techniques may be insufficient to study drug delivery to a particular target site within the skin and should therefore be supplemented with model projections (22) or with other experimental approaches (23) such as high-resolution autoradiography.

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