Effects of Topically Applied Acitretin in Reconstructed Human Epidermis and the Rhino Mouse

Edward Hsia[1], Michael J. Johnston[2], Robert J. Houlden[2], Wendy H. Chern[1] and Hans E.J. Hofland[1]

Oral acitretin is currently indicated for the treatment of severe psoriasis in adults, but its use is limited by systemic side effects and teratogenicity. Topical administration of acitretin may lessen the risk of systemic toxicity while increasing local bioavailability in the skin. The effects of topical acitretin on reconstructed human epidermis (RHE) and Rhino mice were investigated and compared to those of currently marketed topical retinoids: tretinoin and tazarotene. In acitretin-treated RHE cultures, there was a reduction in keratohyalin granules and filaggrin expression in the stratum granulosum, a loss of keratin 10 expression in the stratum spinosum, and an increase in keratin 19 expression in all viable cell layers. All retinoids showed similar signs of activity in RHE cultures. Furthermore, the release of pro-inflammatory cytokines IL-1α and IL-8 in RHE cultures was less pronounced with acitretin compared to tretinoin- and tazarotene-containing formulations, suggesting that acitretin may be less irritating. In Rhino mice, acitretin induced a local, dose-dependent reduction in utricle diameter after seven daily dermal doses. A similar effect was observed in tretinoin- and tazarotene-treated mice. Our data suggest that topical application of acitretin may have a therapeutic benefit in the local management of keratinization disorders.

Journal of Investigative Dermatology (2008) **128,** 125–130; doi:10.1038/sj.jid.5700968; published online 19 July 2007

INTRODUCTION

Retinoids are known to influence keratinocyte differentiation and have proven to be effective in the treatment of a variety of disorders of keratinization (Zouboulis, 2001). The mechanism of action of retinoids, such as all-*trans*-retinoic acid (tretinoin), occurs through their interaction with nuclear retinoic acid receptors (RARs), leading to subsequent gene expression via retinoic acid response elements (Idres *et al.*, 2002). The binding affinity of retinoids to various RAR subtypes (α, β, or γ) is thought to contribute to their relative potency. RARγ is the predominant subtype in human epidermis (Fisher *et al.*, 1994).

Binding of tretinoin to RARs is modulated by the cellular retinoic acid binding proteins (CRABPs): CRABP I and CRABP II (Donovan *et al.*, 1995). CRABP II is expressed in the skin and regulates nuclear levels of tretinoin by sequestration and/or promotion of its metabolism (Boylan and Gudas, 1992; Fiorella and Napoli, 1994). Acitretin, an aromatic synthetic retinoid, has relatively low affinity for RARs, but high affinity for CRABPs (Norris *et al.*, 1994), and has been shown to induce a shift in tretinoin binding from CRABPs to RARγ heterocomplexes (Tian *et al.*, 1997). This suggests that acitretin causes a redistribution of endogenous tretinoin from cytoplasmic to nuclear compartments, thereby indirectly potentiating retinoid activity.

Oral administration of acitretin has been shown to provide therapeutic benefit for the treatment of psoriasis (Lebwohl *et al.*, 2001; Lee and Koo, 2005). It has been marketed for clinical use since 1997 for the treatment of severe psoriasis, and its safety profile is well characterized. It is known to be teratogenic, which limits its use (Kistler and Hummler, 1985; Reiners *et al.*, 1988; Gollnick, 1996).

The clinical efficacy of acitretin following topical application in treating cutaneous diseases is as yet unknown. Percutaneous absorption studies in animals as well as humans have demonstrated that topical application of acitretin can produce dermal concentrations that exceed those achieved with oral administration (Surber *et al.*, 1991, 1993a, b). Topical administration of acitretin may increase the local bioavailability at the site of action (i.e., diseased skin) while minimizing potential systemic exposure; therefore, it would potentially provide a safer treatment for keratinization disorders of the skin.

The main objective of this study was to investigate the potential of acitretin as a topical therapy. Therefore, acitretin activity was benchmarked against commercially available,

[1]*Center for Skin Biology, Stiefel Laboratories Inc., Palo Alto, California, USA and* [2]*Stiefel Research Australia, Melbourne, Victoria, Australia*

Correspondence: Dr Hans E.J. Hofland, Center for Skin Biology, Stiefel Laboratories Inc., 3160 Porter Drive, Palo Alto, California 94304, USA. E-mail: hans.hofland@stiefel.com

Abbreviations: CRABP, cellular retinoic acid binding protein; K10, keratin 10; K19, keratin 19; RAR, retinoic acid receptors; RHE, reconstructed human epidermis; SOA, site of application

Received 21 December 2006; revised 21 May 2007; accepted 23 May 2007; published online 19 July 2007

clinically proven, topical retinoid products. Where possible, direct comparisons between acitretin and other retinoids were made using the same vehicle.

Reconstructed human epidermis (RHE) is a stratified culture that shares histological features that closely resemble human epidermis *in vivo*: basal layer and differentiating spinous, granular, and cornified layers (Poumay *et al.*, 2004). Tretitoin has been shown to induce specific changes in tissue morphology and biomarker expression in RHE and serves as a benchmark for retinoid effects in this model (Rosdy *et al.*, 1997). Moreover, these responses to tretitoin in RHE cultures have been shown to correlate with those observed in normal human skin *ex vivo*, thereby validating the use of RHE as a predictive model for the screening of retinoid activity (Bernard *et al.*, 2002).

The Rhino mouse (RHJ/LeJ) is a strain of the hairless mouse, whose skin contains keratinized pilosebaceous structures (horn-filled utriculi). The Rhino mouse utriculi reduction assay is a well-characterized animal model for the evaluation of retinoid activity (Kligman and Kligman, 1979; Mezick *et al.*, 1984). Topically applied retinoids, such as tretitoin, have been shown to reduce the size of utriculi in the Rhino mouse skin, and this effect is considered predictive of retinoid efficacy (Beehler *et al.*, 1995).

The effects of topical acitretin in RHE and Rhino mouse models were investigated in comparison to topical retinoids with known clinical efficacy and are presented in this study.

RESULTS

Reconstructed human epidermis

Hematoxylin and eosin. Untreated control cultures feature intact basal, spinous, granulous, and cornified cell layers, and the epidermal stratification is orthokeratotic, regular, and normal. Cells in the stratum basale were polarized vertically, and numerous keratohyalin granules were detectable in the stratum granulosum. The RHE cultures treated with acitretin, tretinoin, and tazarotene showed a loss of keratohyalin granules in the stratum granulosum (Figure 1), while vehicle-treated cultures were similar to untreated controls.

Keratin 10. In untreated control cultures, keratin 10 (K10) is expressed in all suprabasal cell layers. K10 expression was lower in the stratum spinosum of acitretin-treated RHE cultures than in untreated controls. A similar decrease in K10 expression was observed in tretinoin- and tazarotene-treated tissues (Figure 2).

Keratin 19. Keratin 19 (K19) is absent in untreated cultures. Treatment with acitretin induced significant expression of K19 in all viable cell layers (Figure 3), although the amount was slightly less than that observed in tretinoin- and tazarotene-treated tissues.

Filaggrin. Filaggrin is normally expressed in the stratum granulosum. Acitretin-treated RHE cultures displayed a decreased expression of filaggrin, as did tretinoin- and tazarotene-treated cultures (Figure 4).

The results described above demonstrate that acitretin modulated epidermal morphology and expression of specific biomarkers of keratinocyte differentiation in a manner similar to that of tretinoin and tazarotene.

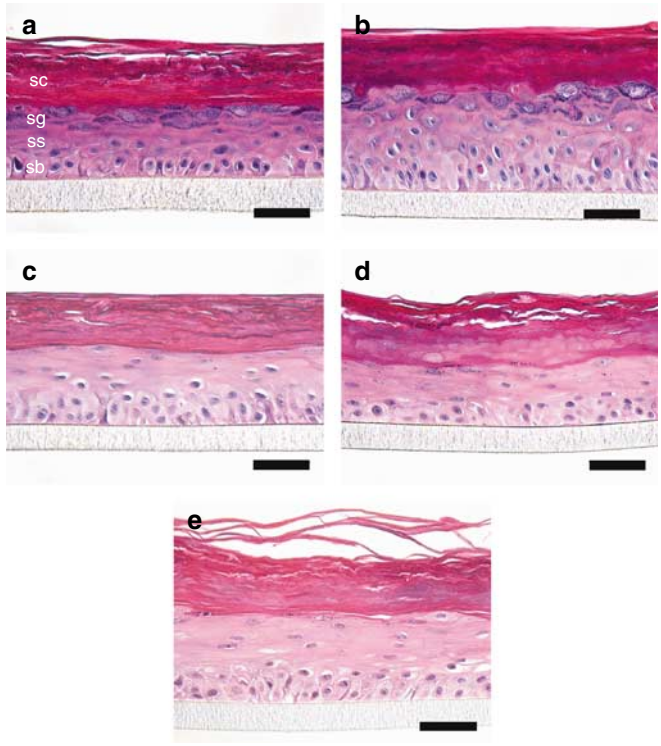

Figure 1. Morphological comparison of RHE tissues treated topically with various retinoid formulations. RHE tissues were (**a**) untreated or treated with (**b**) vehicle, (**c**) acitretin 0.02% solution, (**d**) tretinoin 0.025% cream, or (**e**) tazarotene 0.1% cream, and incubated for 3 days at 37°C. Following this incubation period, tissues were fixed, sectioned, and stained with H&E. Representative micrographs from triplicate cultures are shown. sc, stratum corneum; sg, stratum granulosum; ss, stratum spinosum; sb, stratum basale. Bar = 50 μm.

Cytokine release. The release of IL-1α and IL-8 in the growth media of RHE cultures treated topically with 0.02% acitretin solution was approximately fourfold higher compared to untreated controls ($P<0.01$) (Figure 5). IL-1α and IL-8 release in response to tretinoin and tazarotene was 8- 10-fold higher than that in untreated controls, suggesting that acitretin may be less irritating than tretinoin and tazarotene. The cytokine release in vehicle-treated tissues was similar to that in untreated controls, indicating that the vehicle itself was not irritating.

Rhino mouse model

Once-daily topical administration of 0.2% acitretin solution for 7 days caused a complete (100%) reduction in utricle diameter, which was similar to that observed in tretinoin- and tazarotene-treated mice (Figure 6 and Table 1). Treatment with a lower concentration of acitretin (0.02%) also resulted in a significant reduction of utriculi, but to a lesser degree. In females treated with 0.02% acitretin, utriculi diameter decreased from 90.4 $\pm$ 18.5 μm (sham control) to 2.1 $\pm$ 5.2 μm, whereas in males, utriculi diameter decreased from 123.0 $\pm$ 12.9

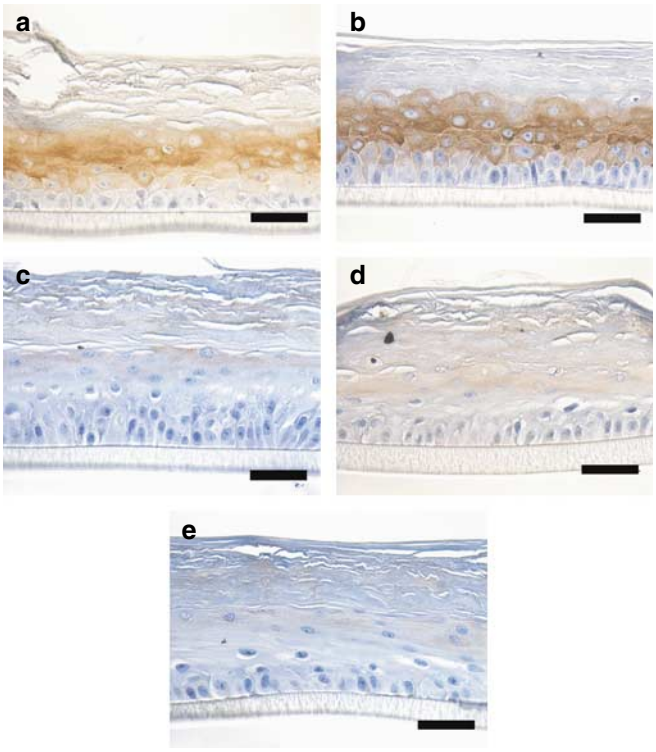

Figure 2. K10 expression in RHE cultures treated topically with various retinoid formulations. RHE tissues were (**a**) untreated or treated with (**b**) vehicle alone, (**c**) acitretin 0.02% solution, (**d**) tretinoin 0.025% cream, or (**e**) tazarotene 0.1% cream, and incubated for 3 days at 37°C. Following this incubation period, tissues were fixed, sectioned, and stained for K10. Representative micrographs from triplicate cultures are shown. Bar = 50 μm.

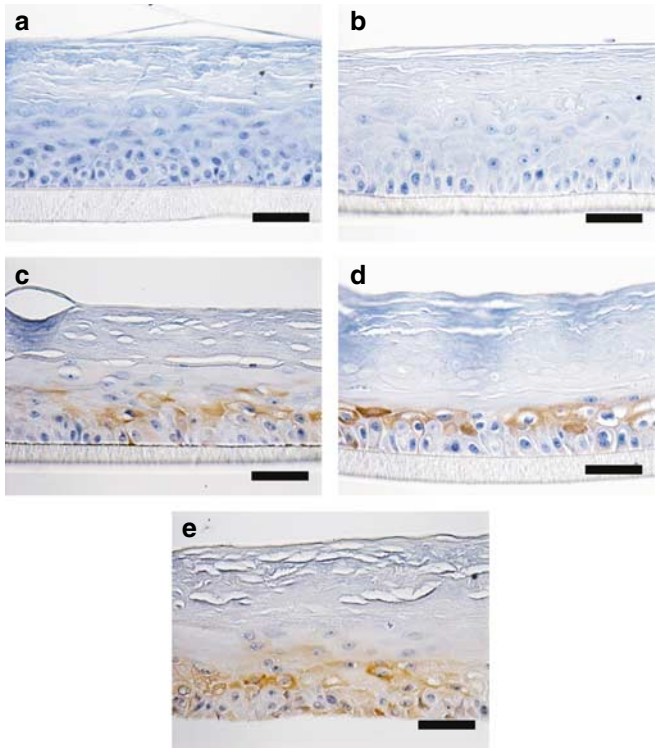

Figure 3. K19 expression in RHE cultures treated topically with various retinoid formulations. RHE tissues were (**a**) untreated or treated with (**b**) vehicle alone, (**c**) acitretin 0.02% solution, (**d**) tretinoin 0.025% cream, or (**e**) tazarotene 0.1% cream, and incubated for 3 days at 37°C. Following this incubation period, tissues were fixed, sectioned, and stained for K19. Representative micrographs from triplicate cultures are shown. Bar = 50 μm.

to 67.6±14.2 μm, representing reductions of 98 and 45% in females and males, respectively. Treatment with vehicle solution alone did not induce a significant reduction in utriculi, demonstrating that the effects of acitretin were specific. Moreover, utricles from skin biopsies taken from nontreated sites were similar to sham controls, indicating that the acitretin effect was local and unlikely to be a result of systemic absorption (Table 1).

DISCUSSION

The cytotoxicity of various solvents in RHE cultures was investigated, and the dose of acitretin used in these experiments (0.02%) was based on its maximum solubility in a non-cytotoxic solvent, octyldodecanol (data not shown). The effects of the tretinoin 0.025% cream formulation on RHE cultures have been well characterized; thus, tretinoin served as a positive control (Bernard *et al.*, 2002). The tazarotene formulation has not been previously demonstrated to induce effects in the RHE model, and served as an additional comparator control. Because tazarotene is a proprietary compound, the concentration and formulation used (0.1% cream) were based on its commercial availability.

Acitretin induced *in vitro* effects in RHE cultures which were comparable to those of tretinoin- and tazarotene-treated cultures. These effects included degranulation, induction of K19 expression, and reduction of K10 and filaggrin. K19 is a non-epidermal keratin that is absent in normal human epidermis. K10 is an early biomarker of epidermal differentiation and is normally expressed in all suprabasal layers (Poumay *et al.*, 1999; Ekholm and Egelrud, 2000). Filaggrin is a marker of late-stage keratinocyte differentiation that is normally expressed in the stratum granulosum. Although the immunohistochemistry data presented are not quantitative, these *in vitro* results suggest that acitretin directly affects differentiating keratinocytes in a manner similar to other topical retinoids with well-established dekeratinizing effects.

The release of pro-inflammatory cytokines, IL-1α and IL-8, in RHE cultures is a well-established biomarker of acute irritation (de Brugerolle de *et al.*, 1999; Medina *et al.*, 2001; Tornier *et al.*, 2006). Topical tretinoin and tazarotene are generally recognized as being clinically effective, but are known to be associated with dermal irritation, which may limit their use. In the RHE model, the acitretin solution appears to be less irritating than the two commercially available products tested (tretinoin and tazarotene creams), suggesting that a topical acitretin product, in a non-irritating vehicle, may strike a more optimal balance between efficacy and irritation in treating disorders of keratinization, while minimizing the risk of systemic adverse effects.

The Rhino mouse bioassay has frequently been used to evaluate the potential efficacy of topical retinoids. Commonly prescribed topical retinoids, such as tretinoin and

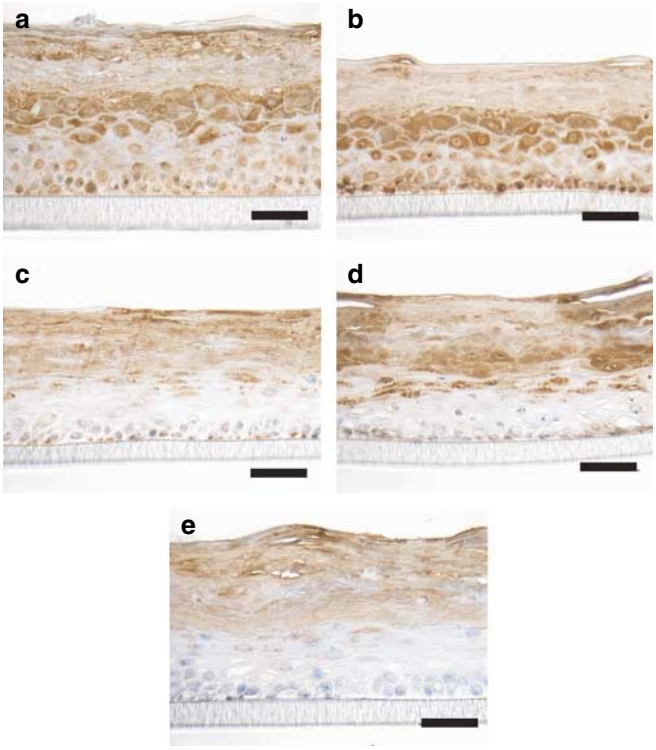

Figure 4. Filaggrin expression in RHE cultures treated topically with various retinoid formulations. RHE tissues were (**a**) untreated or treated with (**b**) vehicle alone, (**c**) acitretin 0.02% solution, (**d**) tretinoin 0.025% cream, or (**e**) tazarotene 0.1% cream, and incubated for 3 days at 37°C. Following this incubation period, tissues were fixed, sectioned, and immunostained for filaggrin. Representative micrographs from triplicate cultures are shown. Bar = 50 μm.

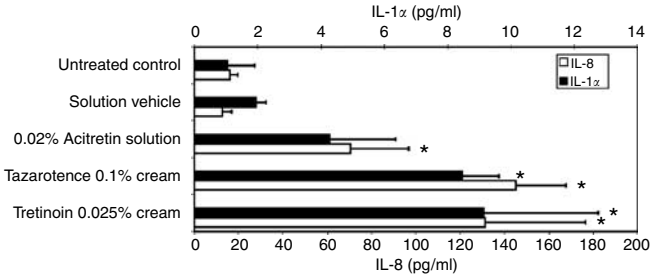

Figure 5. Release of IL-1α and IL-8 from RHE cultures treated topically with various retinoid formulations. Triplicate RHE cultures were treated with various retinoid formulations and incubated for 3 days at 37°C. At the end of this incubation period, concentrations of IL-1α and IL-8 in the media were determined by ELISA. Values are mean ± SD, $*P < 0.05$ (*t*-test) compared to untreated control.

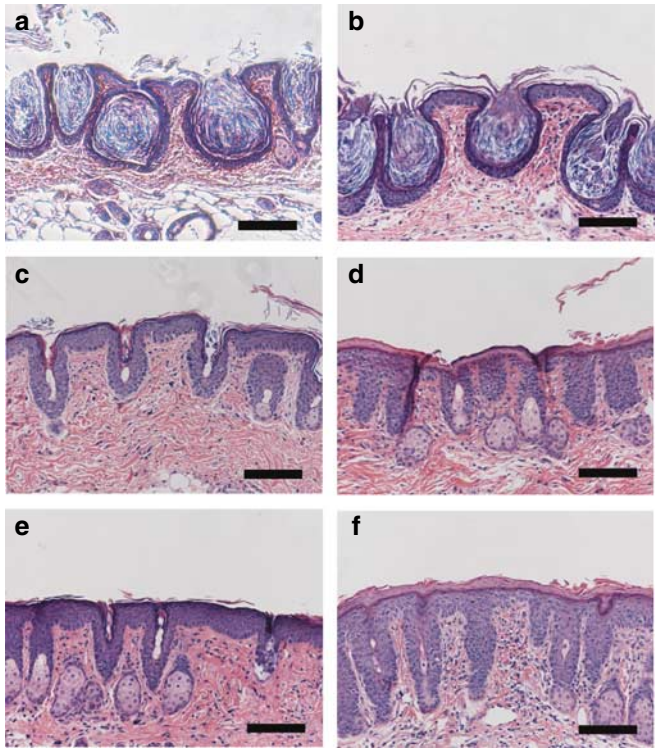

Figure 6. Utriculi of Rhino mice treated topically with various retinoid formulations. Rhino mice ($n = 6$ per group) were dosed topically once daily for 7 consecutive days with (**a**) water, (**b**) vehicle alone, (**c**) acitretin 0.02% solution, (**d**) acitretin 0.2% solution, (**e**) tretinoin 0.025% solution, or (**f**) tazarotene 0.1% cream. Tissues were fixed, sectioned, and stained with H&E. Representative micrographs are shown. Bar = 100 μm.

tazarotene, have shown positive results in the Rhino mouse bioassay in their commercial formulations (Sakuta and Kanayama, 2005). The dermal toxicity of various solvents on Rhino mice was investigated, and the highest dose of acitretin used in these experiments (0.2%) was based on its maximum solubility in a non-toxic solvent, dimethyl isosorbide (data not shown). The lower dose of 0.02% acitretin was chosen in an attempt to demonstrate a concentration-dependent effect. Tretinoin was diluted in the same solvent as acitretin, thus allowing for a direct comparison. The concentration of tretinoin used (0.025%) was chosen on the basis of its clinically effective dose as well as the results of previously published studies using this model (Kligman and Kligman, 1979; Bernerd *et al.*, 1991; Bouclier *et al.*, 1991). Because tazarotene is a proprietary compound, the concentration and formulation used were based on its commercial availability. Tazarotene 0.1% has been previously shown to reduce utriculi diameter in Rhino mice (Sakuta and Kanayama, 2005).

Topical acitretin induced a localized, dose-dependent decrease in utricle diameter in Rhino mice, which was comparable to that of tretinoin- and tazarotene-treated animals. There was no significant increase in epidermal hyperplasia, indicating that dermal inflammation did not significantly contribute to utriculi reduction. Utricle reduction appeared to be sex dependent, which was surprising as this effect has not been reported in previous studies involving male and female Rhino mice (Bouclier *et al.*, 1991). More studies are required to confirm this observation.

Orally administered acitretin has been used for over 10 years in the treatment of severe psoriasis. Although acitretin has been proven to be effective, concerns about potential side effects associated with its systemic absorption have limited its use. Topical administration of acitretin may reduce the risk of systemic exposure, with a potential to increase local efficacy in the treated skin disease area. We

Table 1. Utricle diameter of Rhino mice treated topically with various retinoids

		Utricle diameter (μm)[1]					
	n	**Sham control**	**Solution vehicle**	**Acitretin 0.02% solution**	**Acitretin 0.2% solution**	**Tretinoin 0.025% solution**	**Tazarotene 0.1% cream**
SOA							
Female	6[2]	90.4±18.5	95.5±29.1	2.1±5.2**	0±0**	0±0**	0±0**
Male	6[2]	123.0±12.9	91.4±11.6*	67.6±14.2*	0±0**	0±0**	0±0**
Non-SOA							
Female	3	130.2±18.8	125.9±6.4	98.3±21.0	111.2±10.1	128.6±15.8	116.4±2.4
Male	3	131.4±13.5	109.4±20.4	113.3±6.7	121.8±14.6	132.7±16.6	105.8±31.7

SOA, site of application.
[1]Various retinoid formulations were administered topically once daily for 7 days to Rhino mice (three males and three females per group). Biopsies from the dorsal SOA as well as from nontreated ventral sites (non-SOA) were fixed, sectioned, and stained with H&E. Four measurements of the maximum utricle diameter were taken, averaged, and replicate animal samples averaged with standard deviation calculated. Values are mean±SD.
[2]Two biopsies (caudal and rostral sides) were collected per SOA per animal.
*$P<0.01$ versus sham control, Student's *t*-test.
**$P<0.001$ versus sham control, Student's *t*-test.

demonstrated that acitretin can induce dekeratinizing effects *in vitro* and *in vivo* when applied topically; therefore, it may be effective in treating skin diseases involving abnormal keratinization.

MATERIALS AND METHODS

Reconstructed human epidermis model

RHE cultures were purchased from SkinEthic Laboratories (Nice, France). The cultures were maintained in chemically defined growth medium MCDB 153, containing 5 μg/ml insulin, 1.5 mM calcium chloride, 25 μg/ml gentamycin, and 1 ng/ml EGF. The preparation and characterization of these cultures have been described previously (Rosdy *et al.*, 1997). Briefly, a fully differentiated epidermis was obtained by culturing primary keratinocytes, isolated from normal human foreskin and cultured on an inert microporous polycarbonate filter at the air–liquid interface for 14 days. The following test articles were applied topically at 5 μl/0.5 cm^2 to triplicate RHE tissues and incubated for 3 days at 37°C/5% CO_2: acitretin (Roche, Basel, Switzerland) 0.02% dissolved in octyldodecanol (Cognis, Cincinnati, OH; 0.02% was the maximal amount of acitretin soluble in octyldodecanol), tretinoin cream 0.025% (Ortho Dermatological, Skillman, NJ), and tazarotene cream 0.1% (Allergan, Irvine, CA). Nylon mesh (8 mm diameter) was deposited onto the RHE tissues before application to ensure even spreading of the test articles. Triplicate untreated tissues and acitretin vehicle (octyldodecanol)-treated tissues served as negative controls. This study was performed twice using $n=3$ per group, and the data presented are representative of the two studies.

Cytokine release

Release of IL-1α and IL-8 was quantified in the RHE culture medium using ELISA kits from R&D Systems (Minneapolis, MN).

Rhino mouse model

Male and female Rhino mice (RHJ/LeJ) were purchased from The Jackson Laboratory (West Sacramento, CA). All animal handling was conducted at JAX Services (West Sacramento, CA) in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by The Jackson Laboratory IACUC. Animals were housed individually in static caging in a room with yellow lighting (>500 nm). The mice had access to acidified tap water (pH 2.8–3.2) and Purina Lab Diet 5LL4 *ad libitum* throughout the study. Thirty-six mice, 5–7 weeks of age, were treated once daily for 7 days with the following: acitretin (0.2 or 0.02%) dissolved in dimethyl isosorbide (DMI, Uniqema, New Castle, DE) containing 0.1% butylated hydroxytoluene (BHT, Sigma-Aldrich, St Louis, MO); tretinoin (0.025%) dissolved in DMI containing 0.1% BHT; or tazarotene (0.1%) cream ($n=6$ per group). Sham (water treated) and vehicle controls were included. Coban dressings were applied to the mice after dosing to prevent rub-off and oral ingestion of the test agents. On day 7, 5 hours post-dosing, the skin at the site of application was harvested and cut in half (rostral and caudal sections) for histological evaluation. Ventral tissue (non-SOA) was harvested and used as negative control.

Histology

RHE cultures were fixed in 10% formalin (Richard Allen Scientific, Kalamazoo, MI) and embedded in paraffin. Sections (4 μm) were stained with H&E or using immunohistochemical staining techniques for K10, K19, or filaggrin. Antibodies and staining kits were obtained from Vector Laboratories (Burlingame, CA) and used according to the manufacturer's recommendations.

Rhino mouse skin specimens were fixed in alcohol formalin and embedded in paraffin. Three serial sections (4 μm) spaced 100 μm apart were taken and stained with H&E. Histology was carried out by IDEXX Laboratories (Sacramento, CA).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We acknowledge Sao Kun (Gloria) Ao Ieong for her help with histology, Dr Marina Aliferis of SkinEthic Laboratories (Nice, France) for conducting the RHE studies, and Dr Kara Koehler of The Jackson Laboratory (Sacramento, CA) for conducting the Rhino mouse studies.

REFERENCES

Beehler BC, Chen S, Tramposch KM (1995) Gene expression of retinoic acid receptors and cellular retinoic acid-binding proteins in rhino and hairless mouse skin. *Arch Dermatol Res* 287:488–93

Bernard FX, Pedretti N, Rosdy M, Deguercy A (2002) Comparison of gene expression profiles in human keratinocyte mono-layer cultures, reconstituted epidermis and normal human skin; transcriptional effects of retinoid treatments in reconstituted human epidermis. *Exp Dermatol* 11:59–74

Bernerd F, Demarchez M, Ortonne JP, Czernielewski J (1991) Sequence of morphological events during topical application of retinoic acid on the rhino mouse skin. *Br J Dermatol* 125:419–25

Bouclier M, Chatelus A, Ferracin J, Delain C, Shroot B, Hensby CN (1991) Quantification of epidermal histological changes induced by topical retinoids and CD271 in the rhino mouse model using a standardized image analysis technique. *Skin Pharmacol* 4:65–73

Boylan JF, Gudas LJ (1992) The level of CRABP-I expression influences the amounts and types of all-*trans*-retinoic acid metabolites in F9 teratocarcinoma stem cells. *J Biol Chem* 267:21486–91

de Brugerolle de F, Picarles V, Chibout S, Kolopp M, Medina J, Burtin P *et al.* (1999) Predictivity of an *in vitro* model for acute and chronic skin irritation (SkinEthic) applied to the testing of topical vehicles. *Cell Biol Toxicol* 15:121–35

Donovan M, Olofsson B, Gustafson AL, Dencker L, Eriksson U (1995) The cellular retinoic acid binding proteins. *J Steroid Biochem Mol Biol* 53:459–65

Ekholm E, Egelrud T (2000) Expression of stratum corneum chymotryptic enzyme in relation to other markers of epidermal differentiation in a skin explant model. *Exp Dermatol* 9:65–70

Fiorella PD, Napoli JL (1994) Microsomal retinoic acid metabolism. Effects of cellular retinoic acid-binding protein (type I) and C18-hydroxylation as an initial step. *J Biol Chem* 269:10538–44

Fisher GJ, Talwar HS, Xiao JH, Datta SC, Reddy AP, Gaub MP *et al.* (1994) Immunological identification and functional quantitation of retinoic acid and retinoid X receptor proteins in human skin. *J Biol Chem* 269: 20629–35

Gollnick HP (1996) Oral retinoids – efficacy and toxicity in psoriasis. *Br J Dermatol* 135(Suppl 49):6–17

Idres N, Marill J, Flexor MA, Chabot GG (2002) Activation of retinoic acid receptor-dependent transcription by all-*trans*-retinoic acid metabolites and isomers. *J Biol Chem* 277:31491–8

Kistler A, Hummler H (1985) Teratogenesis and reproductive safety evaluation of the retinoid etretin (Ro 10-1670). *Arch Toxicol* 58:50–6

Kligman LH, Kligman AM (1979) The effect on rhino mouse skin of agents which influence keratinization and exfoliation. *J Invest Dermatol* 73:354–8

Lebwohl M, Drake L, Menter A, Koo J, Gottlieb AB, Zanolli M *et al.* (2001) Consensus conference: acitretin in combination with UVB or PUVA in the treatment of psoriasis. *J Am Acad Dermatol* 45:544–53

Lee CS, Koo J (2005) A review of acitretin, a systemic retinoid for the treatment of psoriasis. *Expert Opin Pharmacother* 6:1725–34

Medina J, Elsaesser C, Picarles V, Grenet O, Kolopp M, Chibout SD *et al.* (2001) Assessment of the phototoxic potential of compounds and finished topical products using a human reconstructed epidermis. *In Vitro Mol Toxicol* 14:157–68

Mezick JA, Bhatia MC, Capetola RJ (1984) Topical and systemic effects of retinoids on horn-filled utriculus size in the rhino mouse. A model to quantify ''antikeratinizing'' effects of retinoids. *J Invest Dermatol* 83: 110–3

Norris AW, Cheng L, Giguere V, Rosenberger M, Li E (1994) Measurement of subnanomolar retinoic acid binding affinities for cellular retinoic acid binding proteins by fluorometric titration. *Biochim Biophys Acta* 1209: 10–8

Poumay Y, Dupont F, Marcoux S, Leclercq-Smekens M, Herin M, Coquette A (2004) A simple reconstructed human epidermis: preparation of the culture model and utilization in *in vitro* studies. *Arch Dermatol Res* 296:203–11

Poumay Y, Herphelin F, Smits P, De Potter IY, Pittelkow MR (1999) High-cell-density phorbol ester and retinoic acid upregulate involucrin and downregulate suprabasal keratin 10 in autocrine cultures of human epidermal keratinocytes. *Mol Cell Biol Res Commun* 2:138–44

Reiners J, Lofberg B, Kraft JC, Kochhar DM, Nau H (1988) Transplacental pharmacokinetics of teratogenic doses of etretinate and other aromatic retinoids in mice. *Reprod Toxicol* 2:19–29

Rosdy M, Bertino B, Butet V, Gibbs S, Ponec M, Darmon M (1997) Retinoic acid inhibits epidermal differentiation when applied topically on the stratum corneum of epidermis formed *in vitro* by human keratinocytes grown on defined medium. *In Vitro Toxicol* 10:39–47

Sakuta T, Kanayama T (2005) Comedolytic effect of a novel RARgamma-specific retinoid, ER36009: comparison with retinoic acid in the rhino mouse model. *Eur J Dermatol* 15:459–64

Surber C, Wilhelm KP, Bermann D, Maibach HI (1993a) *In vivo* skin penetration of acitretin in volunteers using three sampling techniques. *Pharm Res* 10:1291–4

Surber C, Wilhelm KP, Maibach HI (1991) *In-vitro* skin pharmacokinetics of acitretin: percutaneous absorption studies in intact and modified skin from three different species using different receptor solutions. *J Pharm Pharmacol* 43:836–40

Surber C, Wilhelm KP, Maibach HI (1993b) *In vivo* percutaneous absorption of [^{14}C]acitretin in the hairless guinea pig and in the rhesus monkey. *Arzneimittelforschung* 43:1001–4

Tian K, Norris AW, Lin CL, Li E (1997) The isolation and characterization of purified heterocomplexes of recombinant retinoic acid receptor and retinoid X receptor ligand binding domains. *Biochemistry* 36:5669–76

Tornier C, Rosdy M, Maibach HI (2006) *In vitro* skin irritation testing on reconstituted human epidermis: reproducibility for 50 chemicals tested with two protocols. *Toxicol In Vitro* 20:401–16

Zouboulis CC (2001) Retinoids – which dermatological indications will benefit in the near future? *Skin Pharmacol Appl Skin Physiol* 14:303–15