Retinoic Acid Isomers Applied to Human Skin *in Vivo* Each Induce a 4-Hydroxylase That Inactivates Only *Trans* Retinoic Acid

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Application of all-*trans* retinoic acid to human skin for 4 d under occlusion produces a marked increase in retinoic acid 4-hydroxylase activity. In this study, the possible induction of other hydroxylases in response to 9-*cis* and 13-*cis* retinoic acid applications to adult human skin *in vivo* was determined. Application of 0.1% all-*trans*, 0.1% 9-*cis*, and 0.1% 13-*cis* retinoic acid to human skin for 2 d resulted in induction of only all-*trans* retinoic acid 4-hydroxylase activity. The 4-hydroxylase activity in microsomes from the treated tissue ranged from 383 ± 46 to 531 ± 59 pg of 4-hydroxy all-*trans* retinoic acid formed/min/mg protein (n = 6). These same preparations were unable to use 9-*cis* or 13-*cis* retinoic acid as substrate for the hydroxylation reaction. Extraction of the retinoic acid isomers from epidermis 48 h after application of 0.1% solution of each isomer yielded significant amounts of all-*trans* retinoic acid (36–72%) regardless of the isomer applied. The all-*trans* isomer produced by isomerization of both 9-*cis* and 13-*cis* retinoic acids is the likely inducer of the 4-hydroxylase. All-*trans* retinol and all-*trans* retinal were unable to compete with all-*trans* retinoic acid as substrate for 4-hydroxylase enzyme. The 4-hydroxylase induced in response to pharmacological doses of retinoic acids is specific for the all-*trans* isomer. The inability of 9-*cis* or 13-*cis* retinoic acid to induce their own hydroxylation and inactivation or act as substrate for the 4-hydroxylase in skin may have considerable implications in light of the clinical use of retinoids in the treatment of various diseases including cancers. *Key words: metabolism/Isomerization/pharmacology/cytochrome P450.*
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When human [1] or rat skin [2] is treated topically with all-*trans* retinoic acid (*t*-RA), a marked induction of RA 4-hydroxylase occurs. Because this hydroxylase may limit the activity of *t*-RA, 9-*cis* RA, and 13-*cis* RA, we investigated the time course for induction of RA hydroxylase, the specificity of the enzyme with the three isomers of RA, retinol (ROL), and retinaldehyde (RAL) as substrates, and possible induction of other hydroxylases in response to the topical application of 9-*cis* RA or 13-*cis* RA.

MATERIALS AND METHODS

Isocitrate, isocitrate dehydrogenase, NADP, 13-*cis* RA, *t*-RA, 13-*cis* RAL, 9-*cis* RAL, *t*-RAL, 13-*cis* ROL, and *t*-ROL were purchased from Sigma Chemical Co. (St. Louis, MO). Tritiated *t*-RA, and ^{3}H–*t*-ROL were obtained from DuPont NEN (Boston, MA). 9-*cis* RA, 9-*cis* ROL, ^{3}H–9-*cis* RA, ^{3}H–13-*cis* RA were gifts from Drs. Joseph Grippo, Arthur Levin, P.F. Sorter, and A.A. Liebman of Hoffmann La Roche Co. (Nutley, NJ). RA metabolites, 4-OH *t*-RA, 4-oxo *t*-RA, 4-oxo-13-*cis* RA, and 5,6-epoxy RA, were gifts from Drs. Michael Rosenberger and P.F. Sorter of Hoffmann La Roche. All-*trans* RA 0.1% (Retin-A) and cream vehicle were supplied by Ortho Pharmaceutical Corp. (Raritan, NJ). High-performance liquid chromatography (HPLC) grade solvents were used for extraction and chromatographic solvents. Spherisorb ODS-1 columns were obtained from Phase Separations (Norwalk, CT).

Application of Compounds and Biopsy Procedure Solutions of ROL (1.6%), *t*-RA (0.1%), 13-*cis* RA (0.1%) and 9-*cis* RA (0.1%) were prepared in a vehicle consisting of 95% ethanol and propylene glycol (7:3, vol/vol) containing 0.5 mg butylated hydroxytoluene/ml of solution (EPG). The use of EGP as vehicle was required since only *t*-RA was available in cream base used in the initial studies [1]. These solutions and vehicle were applied topically to a 3 × 2–inch area on the buttocks. The area is occluded and kept dark for indicated periods of time, usually 2 or 4 d. The 4-d time point gave consistent clinical evaluations and was the time selected in the initial studies. The 2-d time point, however, is adequate for biochemical studies without clinical correlations. The treated areas were infiltrated with 1% lidocaine local anesthesia and keratomed epidermal tissue (0.1-mm depth) was immediately frozen in liquid nitrogen and stored at −70°C until used. All subjects gave informed, written consent. The study was approved by the University of Michigan Medical Center Institutional Review Board.

Extraction of Retinoids Biopsy sites were tape stripped to glistening prior to biopsy to remove any retinoid trapped in dead stratum corneum that was not absorbed into the viable layers of the skin. Procedures were as previously stated [1]. Briefly, frozen keratome biopsies were ground to a powder under liquid nitrogen with a mortar and pestle. The material was quickly transferred to a homogenizer containing the extraction solvents (chloroform and methanol [2:1]) with tracer ^{3}H–*t*-RA (to estimate recoveries) and was homogenized. After two additional extractions, the organic phase was evaporated, resuspended, filtered, and injected onto the HPLC column. Protein determinations were by the method of Lowry *et al* [3] with bovine serum albumin as a standard.

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Abbreviations: *t*-RA, all-*trans* retinoic acid; 9-*cis* RA, 9-*cis* retinoic acid; 13-*cis* RA, 13-*cis* retinoic acid; ROL, retinol; RAL, retinaldehyde; EPG, 95% ethanol: propylene glycol (7:3, vol/vol) with 0.5 mg butylated hydroxytoluene/ml.

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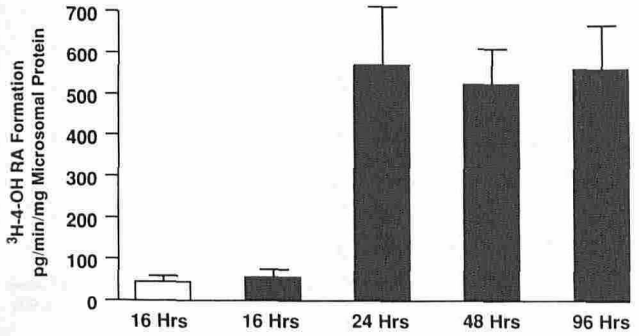

Figure 1. Time course for induction of RA-4 hydroxylase activity after topical application of 0.1% t-RA cream to adult human skin under occlusion. ^{3}H–*t*-RA (2 μM) and an NADPH regenerating system was incubated *in vitro* with microsomes from tissues treated *in vivo* with *t*-RA cream. Other assay conditions and separation of metabolites are given in *Materials and Methods*. □, vehicle; ■, samples from *t*-RA sites treated for the times indicated. The number of individual subjects from whom microsomes were prepared at the indicated time points are as follows: n = 6 for vehicle and *t*-RA treated for 16 and 24 h; n = 8 for 48 h; n = 5 for 96 h. *Error bars*, SEM, for each series.

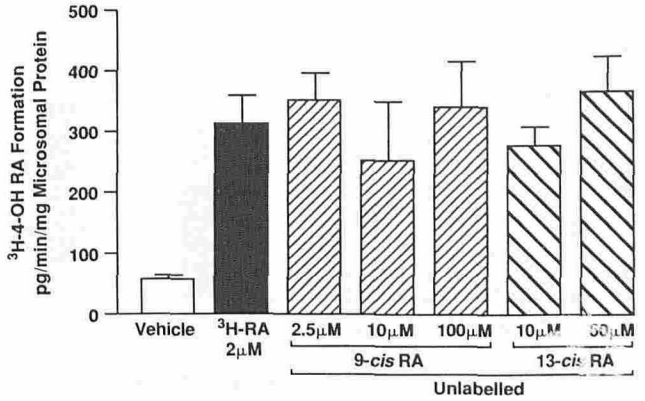

Figure 2. 9-*cis* RA and 13-*cis* RA isomers of *t*-RA are not substrates for RA 4-hydroxylase activity induced by topical application of 0.1% *t*-RA cream. Assay conditions are given in legend to **Fig 1** except for the addition of the unlabeled isomers. The number of microsomal fractions used are as follows: vehicle (n = 3), and ^{3}H–*t*-RA (n = 13); for added 9-*cis* RA—2.5 μM (n = 7), 10 μM, (n = 3), and 100 μM (n = 6); for added 13-*cis* RA—10 μM (n = 3) and 60 μM (n = 6). *Error bars*, SEM. Using Student's t test for paired samples, there was no significant difference in 4-OH RA formation in the presence or absence of RA isomers ($p > 0.5$).

Enzyme Assays Microsomal fractions were prepared as previously described [1]. RA and RA metabolites were kept in amber glass in darkened rooms with yellow lighting. The rate of RA metabolism was determined by incubating 100 μg microsomal protein in 0.01 M phosphate buffer, pH 7.4, containing an NADPH regenerating system and ^{3}H–*t*-RA or other tritiated retinoids as substrates. Samples were incubated for 30 min at 35°C. The activity is expressed as picograms of 4-OH RA formed per minute per milligram of microsomal protein. Compounds tested as possible competitive substrates were added to the assay mix just prior to the addition of ^{3}H–*t*-RA. The reaction was terminated by addition of 100 μl of methanol cooled to −20°C.

HPLC Separation An HPLC system (1090M; Hewlett-Packard Co., Palo Alto, CA) containing a Spherisorb *ODS-1* column (25 × 4.6 mm), a diode array detector and a chem workstation was used to separate and quantitate retinoids and metabolites [1]. The standard compounds used to calibrate the column contained the following retinoids: 4-OH RA; 4-oxo-13-*cis* RA; 4-oxo *t*-RA; 5,6 epoxy RA; 13-*cis* RA; 9-*cis* RA; *t*-RA; 13-*cis* ROL; *t*-ROL; 13-*cis* RAL; *t*-RAL. A gradient elution system separated the retinoids and metabolites in a 42-min time period. Effluent from the HPLC column flowed directly into a flow through scintillation counter (Flo-One Beta model 295A, Packard Instrument Co., Meriden, CT) with a computerized data capture system to determine radioactivity in each peak. The amount of metabolite formed was calculated based on the specific activity of added [^{3}H]retinoid.

RESULTS

Time Course of RA 4-Hydroxylase Induction in Microsomes Isolated from Human Skin Following *In Vivo* Application of 0.1% *t*-RA A marked induction of *t*-RA 4-hydroxylase was reported after human skin had been exposed to 0.1% *t*-RA continuously for 4 d under occlusive patch [1]. **Figure 1** shows that in comparison to cream base only (45 ± 15 pg/min/mg microsomal protein), no RA 4-hydroxylase induction occurred at 16 h after application of *t*-RA in cream base (56 ± 18 pg/min/mg protein). In data not shown, there is no significant difference in 4-hydroxylase activity in cream base only treated areas in comparison to areas receiving no treatment. The baseline RA 4-hydroxylase activity varies with different individuals from barely detectable to moderate activity. Twenty-four hours after a single application of *t*-RA in cream base, a 10-fold induction of RA 4-hydroxylase activity (569 ± 142 pg/min/mg protein) had occurred. Similar activities were maintained 48 or 96 h after application of *t*-RA, as shown in **Fig 1**. The time lag of greater than 16 h but less than 24 h for the increase in RA 4-hydroxylase activity includes the time required for penetration of the *t*-RA, activation of gene transcription, and mRNA and protein synthesis.

9-*cis* RA and 13-*cis* RA as Substrates for RA-4-Hydroxylase in Skin To test whether or not the all-*trans* configuration was necessary for RA to be a substrate for the induced enzyme, nonradioactive 9-*cis* and 13-*cis* RA were added as competitive substrates for the ^{3}H–*t*-RA in the standard *in vitro* assay. The data are given in **Fig 2**. The ^{3}H–*t*-RA concentration was 2 μM with 4-OH *t*-RA formation of 315 ± 46 pg/min/mg protein. The addition of nonradioactive 2.5 μM 9-*cis* RA, 10 μM 9-*cis* RA, or 100 μM 9-*cis* RA did not significantly decrease the amount of ^{3}H–4-OH *t*-RA formed (average of 317 pg/min/mg protein for all 9-*cis* RA concentrations). **Figure 2** also shows that similar results were obtained if nonradioactive 10 μM 13-*cis* RA or 60 μM 13-*cis* RA were present in the assay (average of 323 pg/min/mg protein for both concentrations of 13-*cis* RA). Thus, neither 9-*cis* RA nor 13-*cis* RA were able to compete with *t*-RA as substrate for the enzyme reaction.

In contrast, **Fig 3** shows that the addition of unlabeled *t*-RA to 2 μM ^{3}H–*t*-RA decreased the DPM present in the 4-OH *t*-RA and this decrease was proportional to the decrease in specific activity of the ^{3}H–*t*-RA. Based on the specific activity of the substrate, there is no change in enzymatic activity (751 ± 122 pg/min/mg protein

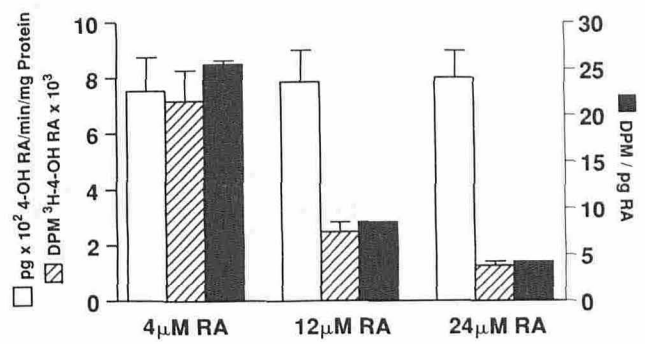

Figure 3. Addition of unlabeled *t*-RA decreases the formation of ^{3}H–4-OH *t*-RA but not the quantity of 4-OH *t*-RA. Assays were as given in legend to **Fig 1** except additional unlabeled *t*-RA was added. Formation of 4-OH *t*-RA (pg) was calculated based on specific activity of added substrate. Microsomes were prepared from human skin treated *in vivo* with 0.1% *t*-RA cream (n = 3). *Error bars*, SEM.

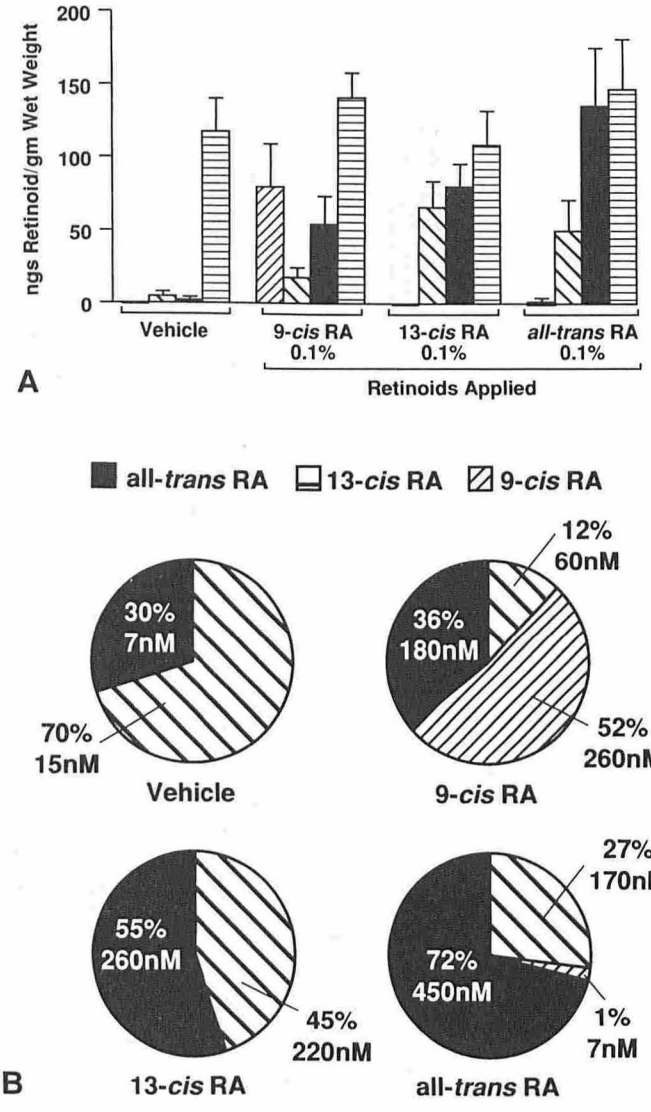

Figure 4. Quantities of 9-*cis* RA, 13-*cis* RA, and *t*-RA extracted from human skin following *in vivo* application in EPG of 0.1% of each isomer for 48 h at different sites on the same individuals. *A*) Nanograms per gram wet weight of each RA isomer and *t*-ROL extracted from the skin. The stratum corneum was stripped prior to keratoming. Extracted material from treated areas: vehicle (□); 9-*cis* RA (▨); 13-*cis* RA (▧); with *t*-RA (■); ROL (▤). *Error bars*, SEM (n = 7). *B*) The percentage of each isomer was calculated based on the sum of nanograms of extracted RAs from each site as 100%. One gram of tissue = 1 ml was used to calculate nanomolars.

at 4 μM RA, 785 ± 112 pg/min/mg protein for 12 μM RA, and 800 ± 96 pg/min/mg protein for 24 μM RA).

Extraction of RA Isomers Present in Epidermis 48 h After *in Vivo* Application of Vehicle, 9-*cis* RA, 13-*cis* RA and *t*-RA to Human Skin All three RA isomers are soluble in EPG at a concentration of 0.1%. Two days after application of vehicle (EPG) and the three RA isomers in EPG to four different sites under occlusion on the buttock, the stratum corneum (outer layers of the skin) was removed by tape stripping. This was necessary in order to avoid erroneously high values for RA content in the viable layers due to unabsorbed material remaining on the surface of the skin.

Retinoic acids were extracted from the stripped, keratomed tissue and the amount of each RA isomer determined. These data are presented in **Fig 4*A*,*B***. The bar graph in **Fig 4*A*** displays the

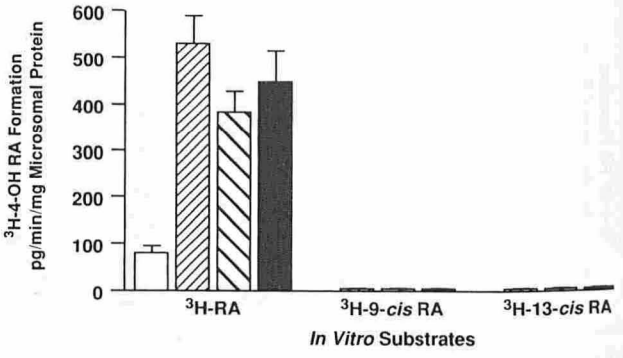

Figure 5. All-*trans* RA 4-OHase activity induced in human skin 48 h after *in vivo* application of 9-*cis* RA, 13-*cis* RA, or *t*-RA. Unlabeled RA isomers were applied *in vivo* in EPG solution for 48 h. All three tritiated isomers were used as substrates in the *in vitro* assay. Assay procedures given in legend to **Fig 1** and in *Materials and Methods*. *Error bars*, SEM, for seven different microsomal preparations: vehicle (□); with 9-*cis* RA (▨); with 13-*cis* RA (▧); with *t*-RA (■).

nanograms per gram wet weight of each RA isomer extracted from each of the treated areas. The sum of the nanograms for the three RA isomers for each treated site is defined as 100%. The individual isomers extracted from a given site are expressed as a percentage of the sum and displayed in pie chart form in **Fig 4*B***. Molar concentration of each RA isomer is also given for the four treated sites in **Fig 4*B***. The total nanograms of the three isomers of RA extracted from the epidermis at each treated site were similar (145–188 ng RAs/g wet weight of tissue). All-*trans* RA (*solid bar*) was the most stable isomer with 72% of the applied isomer remaining as *t*-RA and achieved a concentration within epidermis of 0.45 μM. A significant portion of total retinoic acids extracted from the tissue was *t*-RA regardless of the isomer applied (36% in 9-*cis* RA application areas, 55% in 13-*cis* RA application areas, and 72% in *t*-RA application areas). 9-*cis* RA (*narrow hatched bars*) was recovered from 9-*cis* RA–treated areas (52%) and from *t*-RA–treated areas (1%), but none was detected in the 13-*cis* RA–treated areas. 13-*cis* RA (*wide hatched bars*) was recovered from all of the treated areas.

Induction of 4-Hydroxylase Activity in Epidermis 48 h After *in Vivo* Application of Vehicle, 9-*cis* RA, 13-*cis* RA, and *t*-RA to Human Skin In a second series, the same solutions of the three RA isomers were applied and kept occluded for 2 d. Since RA isomers remaining on the surface would not interfere with either the microsomal preparation or the *in vitro* assay, the stratum corneum was not removed by tape stripping prior to the keratome procedure. The results of the *in vitro* assays for RA 4-hydroxylase activity are depicted in **Fig 5**. With ^{3}H–*t*-RA as substrate, 4-OH *t*-RA was formed by microsomal fractions from areas treated with each of the three isomers. The activity associated with 9-*cis* RA treated areas was 531 ± 59 pg/min/mg protein, with 13-*cis* RA 349 ± 68 pg/min/mg protein and with *t*-RA 448 ± 68 pg/min/mg protein (n = 7). The vehicle value was 70 ± 16 pg/min/mg protein (n = 7). With ^{3}H–9-*cis* RA or ^{3}H–13-*cis* RA as substrate, no hydroxylated products were formed since no new peaks of radioactivity were detected in the region where more polar compounds elute (i.e., compounds with retention times earlier than 13-*cis* RA [28 min]).

Application of *t*-ROL Increases RA 4-Hydroxylase Activity A solution of 1.6% *t*-ROL in EPG was applied to adult human skin for 4 d under occlusion. Microsomal fractions were prepared from these treated areas and were incubated *in vitro* with ^{3}H–*t*-ROL or ^{3}H–*t*-RA as substrate. As shown in **Fig 6**, the application of *t*-ROL or *t*-RA induced the *t*-RA 4-hydroxylase activity based on the *in vitro* assay with ^{3}H–*t*-RA as substrate. All-*trans* RA was a much

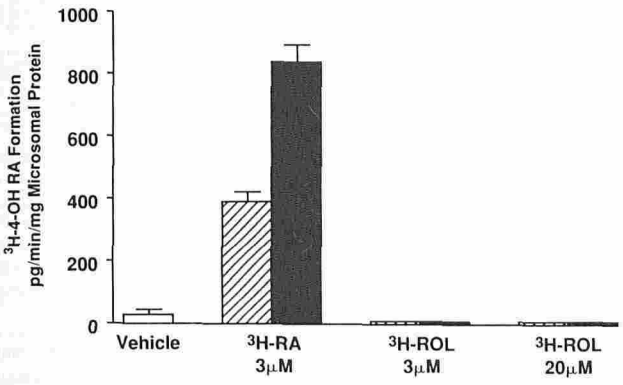

Figure 6. All-*trans* RA 4-OHase activity induced in human skin 96 h after application of 1.6% *t*-ROL or 0.1% *t*-RA. Tritiated *t*-RA or *t*-ROL were substrates in the assay of microsomes obtained from areas treated *in vivo* with *t*-RA or *t*-ROL in EPG solution. *Error bars*, SEM (n = 5, for ^{3}H–*t*-ROL as substrate; n = 4, for ^{3}H–*t*-RA as substrate). □, vehicle; ■, 0.1% *t*-RA; ▨, 1.6% *t*-ROL.

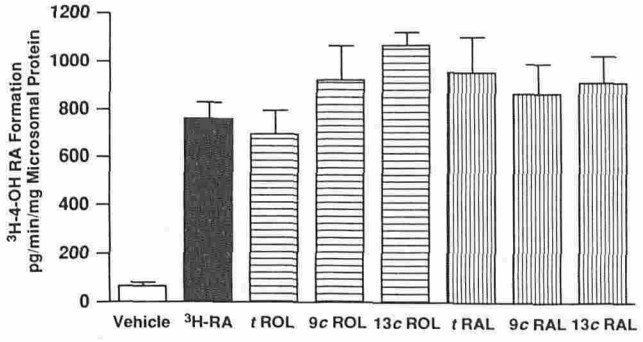

Figure 7. Three isomers of ROL and RAL do not compete with the transformation of ^{3}H–*t*-RA to 4-OH RA *in vitro* with microsomes obtained from areas exposed to 0.1% *t*-RA cream *in vivo* for 96 h. Assays components as given in legend to **Fig 1**. Unlabeled 9-*cis*, 13-*cis*, and all-*trans* isomers of retinol (20 μM) and retinal (20 μM) were added to the *in vitro* assay in a 7-fold excess of the substrate ^{3}H–*t*-RA. *Error bars*, SEM (n = 18, for assays with vehicle or ^{3}H–*t*-RA alone; n = 6 for all other experiments given).

more efficient inducer of RA 4-hydroxylase (841 ± 58 pg/min/mg microsomal protein, n = 4) in comparison to *t*-ROL (391 ± 32 pg/min/mg protein, n = 5). The rate of hydroxylation of *t*-RA in microsomes from vehicle-treated areas was 28 ± 17 pg/min/mg protein (n = 4). When microsomal fractions prepared from the ROL-treated areas were assayed for ROL hydroxylase activity, there was no formation of ^{3}H–4-OH ROL since no radioactive peaks were detected at retention times consistent with polar metabolite formation even at a substrate concentration of 20 μM ^{3}H–*t*-ROL. Although 4-OH ROL can be oxidized to additional metabolites in other tissues, no unidentified peaks were present in the chromatograms from these incubations.

Other retinoids tested for their capacity to function as alternative substrate were the other two ROL isomers and the three corresponding RAL isomers. These retinoids (20 μM) were added to the standard *in vitro* assay containing ^{3}H–*t*-RA, which resulted in a 7-fold excess of ROLs or RALs compared with ^{3}H–*t*-RA. The microsomes used in the *in vitro* assays were obtained from areas treated with 0.1% *t*-RA. As shown in **Fig 7**, there was a 10-fold induction of 4-hydroxylase activity 765 ± 67 pg/min/mg microsomal protein (n = 18) for the *t*-RA treated areas compared with 63 ± 14 pg/min/mg protein (n = 18) for vehicle-treated areas. There were no significant decreases in the amount of ^{3}H–4-OH *t*-RA formed by the addition to the assay of any retinol or retinal isomer. The activity in the presence of 20 μM *t*-ROL or *t*-RAL was 700 ± 101 pg 4-OH *t*-RA/min/mg protein (n = 6) or 958 ± 148 pg/min/mg protein (n = 6). These values are not significantly different from *t*-RA alone.

DISCUSSION

The clinical use of retinoids *t*-RA and 13-*cis* RA in topical preparations circumvents one of the cell's control points (the rate of synthesis of RAs) for maintaining the necessary concentration of *t*-RA. Metabolism of the added retinoids to less active metabolites is one of the mechanisms that can be used to limit effects of topically applied retinoids. While little is known about the specifics of retinoid metabolism and inactivation in skin, the induction of a 4-hydroxylase activity in response to topical application of *t*-RA is known [1,2]. A major metabolite of 13-*cis* RA found in blood following oral administration is 4-oxo-13-*cis* RA [4], a stable 4-hydroxylated metabolite of 13-*cis* RA. *In vitro* incubation of 13-*cis* RA with a 9,000*g* supernatant fraction from rat liver produced the same metabolite [5]. Hydroxylation maybe the main mechanism for inactivation of RAs. The possibility existed that the cytochrome P450 4-hydroxylase induced in response to *t*-RA in skin might also hydroxylate the other isomers (i.e., 9-*cis* RA and 13-*cis* RA) in skin.

The data demonstrate that the 4-hydroxylase induced following application of *t*-RA is specific for *t*-RA. Since retinoids are known to isomerize with exposure to air and light, the stability of each isomer during penetration into the viable layers of the skin was determined. The results show that *t*-RA was the most stable of the three isomers and that regardless of the isomer of RA applied, at least 40–50% of the RA in the viable layers of the epidermis is isomerized to *t*-RA. These data are consistent with recent data obtained with rat liver microsomes *in vitro* [6]. In the report, 40–50% of the added 13-*cis* RA was converted into *t*-RA after a 30-min incubation at 37°C whereas only 11% of added *t*-RA was isomerized into 13-*cis* RA.

The isomerization of the applied 9-*cis* RA and 13-*cis* RA to *t*-RA in the skin would explain the observed induction of the *t*-RA 4-hydroxylase in the epidermis regardless of the isomer applied. A sufficient level of *t*-RA was formed by isomerization of 9-*cis* and 13-*cis* RA so that the same results were produced as if *t*-RA had been applied (i.e., induction of *t*-RA 4-hydroxylase). It is also important to note that neither 9-*cis* RA nor 13-*cis* RA were able to induce a hydroxylase activity in skin that would result in inactivation of these isomers.

Liver, however, does appear able to metabolize 13-*cis* RA by hydroxylation. Experiments with mice showed that feeding 13-*cis* RA increased elimination of 13-*cis* RA only in liver, whereas feeding *t*-RA increased the elimination of *t*-RA in all tissues studied [7]. In other studies microsomal preparations from mouse liver but not mouse skin were able to hydroxylate 13-*cis* RA in an *in vitro* assay system [8].

In human liver cytochrome P450 2C8 hydroxylates not only *t*-RA but also *t*-ROL [9] and rabbit liver P450 2B4 and 1A2 hydroxylate both *t*-ROL and *t*-RAL [10]. Both of these studies were carried out with purified cytochrome P450s. Since topical application of ROL to adult human skin produces many of the same effects as RA [11], we determined whether *t*-ROL could induce a *t*-ROL and/or *t*-RA 4-hydroxylase. Since conversion of ROL to RA may be necessary for induction of RA 4-hydroxylase activity, a much higher concentration of ROL was used to assure adequate penetration and conversion to RA. Topical application of 1.6% ROL, a concentration 16-fold > *t*-RA, did induce RA 4-hydroxylase activity, but to a lesser extent, 46.5% of that achieved with 0.1% *t*-RA. In contrast to the P450s in liver, however, the skin P450 induced by *t*-ROL did not hydroxylate *t*-ROL as indicated by a lack of conversion of ^{3}H-ROL to more polar compounds in the *in vitro* assay system. While 4-OH ROL is less stable than 4-OH RA, the formation of 4-OH ROL with skin as the enzyme source should have been detected if formed since *in*

vitro assay system was similar to that used to detect 4-OH ROL with liver as the enzyme source [9,10].

The skin appears to tightly control its *t*-RA levels by strict limitation of its formation from ROL and by specific inactivation through 4-hydroxylation. Skin does not appear, however, to have a direct means of controlling 13-*cis* or 9-*cis* RA levels after topical application, other than isomerization to *t*-RA or possibly glucuronidation.

Inactivation of applied 13-*cis* RA could be absorption into the blood stream and transport to the liver for conversion to 4-OH-13-*cis* RA by only cytochrome P4502C8 found in liver [9]. Such an inactivation, however, if it occurs in liver, would not be clinically important because topical application of *t*-RA and 13-*cis* RA in man produces slight and variable changes in blood levels of these retinoids [12,13]. Therefore, local inactivation in the skin of topically applied, naturally occurring stereoisomers of RA via a final common isomerization pathway to *t*-RA and subsequent 4-hydroxylation appears to be a critical detoxification mechanism in skin. At the present time little is known about the which P450 associated enzyme carries out this reaction in skin. The use of substrates and inhibitor specific for a P450 enzyme family has not shed light on this question (unpublished data). Likewise, the presence of CRABP I or II in the assay did not alter the rate of RA 4-hydroxylation (unpublished results).

Lastly, the kinds of experiments described in this article are difficult to perform in noncutaneous tissues. Nonetheless, should other tissues be similar to skin in their capacity to convert 9- and 13-*cis* RA to *t*-RA for inactivation, the mechanisms described here may have considerable implications for the treatment of systemic diseases such as aerodigestive tract cancer and acute promyelocytic leukemia with the natural isomers of RA [14,15].

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