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Retinoic Acid 4-Hydroxylase Inducibility and Clinical Response to Isotretinoin in Acne Patients

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Abstract

Background—The cytochrome P450 enzyme CYP26 (retinoic acid 4-hydroxylase) initiates the catabolism of all-*trans* retinoic acid (*t*RA) and limits the effects of *t*RA. The CYP26 enzyme acts on *t*RA, but not 13-*cis* RA (isotretinoin), a retinoid used to treat severe acne. However, 13-*cis* RA can isomerize to *t*RA, which can then be metabolized by CYP26.

Objective—In healthy subjects, we assessed the variability of CYP26 enzymatic activity. We then investigated whether response to oral 13-*cis* RA among acne patients correlates with variability in CYP26 expression.

Methods—In healthy subjects, we isolated microsomal fractions from the epidermis of keratome biopsies and measured CYP26 enzymatic activity in untreated skin and skin treated with *t*RA. Enzymatic activity was determined based on rate of formation of 4-hydroxy RA (pg/min) per mg microsomal protein. Using real-time PCR we quantified CYP26 mRNA induction after *t*RA application in acne patients who responded or did not respond to one course of 13-*cis* RA.

Results—In normal skin (N=118), CYP26 enzymatic activity was widely variable (1–180 pg/min per mg microsomal fraction; mean 42.7 ± 3.5). Furthermore, CYP26 enzymatic activity was inducible in a dose-dependent manner in normal skin following *t*RA application, but not correlated with age or sex (N=29). In acne patients, CYP26 mRNA induction following 0.1% *t*RA application did not differ (P>0.05) between subjects who responded (N=8, 587±325 fold) or did not respond (N=8, 657±227 fold) to one course of 13-*cis* RA.

Limitations—The small number of acne patients treated with 13-*cis* RA was a major limitation.

Conclusion—Factors other than CYP26 activity may determine response to isotretinoin in acne.

Keywords

cytochrome; retinoic acid 4-hydroxylase; isotretinoin

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INTRODUCTION

Retinoids influence various cellular processes in the skin, such as keratinocyte and sebocyte differentiation. Some well-known retinoids include 13-*cis* retinoic acid (isotretinoin, 13-*cis* RA) and its stereoisomers, all-*trans* retinoic acid (tretinoin, *t*RA) and 9-*cis* retinoic acid (alitretinoin, 9-*cis* RA). Many of the biological effects of retinoids are mediated by two families of receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). These receptors function as dimeric, ligand-dependent transcription factors, indicating that retinoids elicit biological effects in part by altering gene expression. Whereas *t*RA and 9-*cis* RA bind to RARs and RXRs, respectively, 13-*cis* RA binds with little or no affinity to either type of receptor.[1,2] Instead, 13-*cis* RA appears to exert many of its effects by first isomerizing to *t*RA.[3] Thus, 13-*cis* RA acts as a prodrug that modulates cellular function via *t*RA-RAR interactions.

In humans, the level of *t*RA is regulated by a feedback system. Cytochrome P450 (CYP) enzymes are primarily responsible for *t*RA catabolism. One of these enzymes, known as CYP26 or RA 4-hydroxylase, initiates the process of *t*RA catabolism and is thus crucial for limiting the biological activity of *t*RA.[2] CYP26 metabolizes *t*RA to less active derivatives, including 4-hydroxy RA and 4-oxo RA[4], but does not directly metabolize other retinoids, such as 9-*cis* RA or 13-*cis* RA.[2] Rather, these *cis*-retinoids are thought to isomerize to *t*RA before being acted upon by CYP26.[5] Furthermore, CYP26 has a retinoic acid response element (RARE) and is selectively induced by *t*RA.[2] Following exposure of human skin to 0.1% *t*RA cream, enzymatic activity of CYP26 is induced substantially.[5] Thus, CYP26 is induced by *t*RA and also targets *t*RA for inactivation. Additionally, we have previously found that gene expression of CYP26 correlates with its enzymatic activity.[2]

Clinically, oral 13-*cis* RA (isotretinoin) is the most effective treatment for severe nodulocystic acne. While the exact biological mechanism of this drug remains unclear, 13-*cis* RA is effective in most (up to 89%) acne patients.[6] However, approximately 16% of treated subjects require a second course.[7] One analysis demonstrated that 70% of patients under 12 years treated with 13-*cis* RA relapsed within 1 year and required a second course of treatment, as compared to 34.8% of patients between 14 to 16 years old.[8]

Since genetic polymorphism exists for CYP26,[9] we hypothesized that patients whose acne did not clear after one course of 13-*cis* RA (defined as “non-responders”) may have higher baseline levels of CYP26 or greater ability to induce CYP26 than patients who responded to one treatment course (defined as “responders”). Our reasoning was that upregulated CYP26 expression in non-responders may increase the catabolism of retinoids, diminishing the levels of active *t*RA derived from 13-*cis* RA isomerization. To investigate this, we first measured the range of CYP26 enzymatic activity in normal human skin and confirmed the inducibility of this enzyme by *t*RA. To investigate mechanisms underlying the improvement of acne with isotretinoin treatment, we then measured the inducibility of CYP26 in the skin of acne patients who were previously treated with this drug.

MATERIALS & METHODS

Subjects

This study was approved by the University of Michigan Medical School Institutional Review Board and conducted in accordance with the Helsinki Declaration. The study was conducted in the Program for Clinical Research in Dermatology (PCRID). Written informed consent was obtained from subjects prior to enrollment. Healthy subjects were enrolled to investigate normal CYP26 expression (N=118) and CYP26 induction by *t*RA (N=29). “Healthy” subjects were defined as not having any skin disease. In addition, we recruited

acne patients (N=16) who had previously completed one course of 13-*cis* RA. Exclusion criteria included age less than 18 at the time of enrollment, application of topical corticosteroids or retinoids within 14 days, or use of systemic corticosteroids within 28 days or systemic retinoids within 3 months. Pregnancy and lactation were also exclusionary.

Retinoic Acid Application and Biopsies

Trans-RA was dissolved in a vehicle consisting of 95% ethanol-propylene glycol (7 parts ethanol to 3 parts propylene glycol by volume) containing 0.5 mg butylated hydroxytoluene per ml of solvent (Sigma, St. Louis, MO). All work with *t*RA was conducted under subdued yellow light. In healthy subjects, no treatment was given, or vehicle alone (100 μL) and up to six concentrations of *t*RA (0.001%, 0.005%, 0.01%, 0.025%, 0.05%, and 0.1%, each 100 μL) were applied under occlusion to different areas of buttock skin for 24 hours. Keratome biopsies (1 inch by 3 inches) were obtained as previously described.[10]

In acne patients previously treated with 13-*cis* RA, vehicle (35 μL) and 0.1% *t*RA (35 μL) were applied to different areas (each 1 in^2) of buttock skin. Skin was occluded with plastic wrap and shielded from light with gauze. After 24 hours, punch biopsies (4 mm) of treated and control skin were obtained after injecting local anesthesia (lidocaine). Samples were snap frozen in liquid nitrogen and stored at -70° C for later analysis.

CYP26 Enzymatic Activity

CYP26 enzymatic activity in keratome samples was determined by measuring the formation of 4-hydroxy RA (pg/min per mg microsomal protein) in microsomal fractions that were isolated and prepared from human epidermis, as previously described.[5,10] Briefly, CYP26 activity was determined in an *ex vivo* assay by incubating 100 μg microsomal protein in 0.01 M phosphate buffer (pH 7.4) containing tritiated *t*RA as substrate (DuPont NEN, Boston, MA) and an NADPH-regenerating system (Sigma, St. Louis, MO).[10] Samples were incubated for 30 minutes at 35°C. The reaction was then terminated by adding 100 μL methanol containing 100 μg/mL of butylated hydroxytoluene cooled to -20°C. After centrifugation at 1000 *g* for 10 minutes, the supernatant fractions were analyzed for tritiated 4-hydroxy RA by reverse phase HPLC and liquid scintillation spectrometry. Due to the limited amount of microsomal protein available, this technique was performed once per biopsy.

Measurement of CYP26 mRNA Levels

In biopsies of skin from acne patients, total RNA was extracted using a commercial RNeasy Mini kit (Qiagen, Chatworth, CA), and reverse transcriptase, real-time polymerase chain reaction (RT-PCR) was performed, as previously described.[11] CYP26 mRNA levels were normalized to an internal control, the housekeeping gene *36B4*. Primers and probes for CYP26 and 36B4 were produced by a custom oligonucleotide synthesis service (Applied Biosystems, Foster City, CA).

Statistical Analysis

Comparison of CYP26 induction levels following application of varying concentrations of *t*RA was performed with a repeated measures analysis of variance and Dunnett's multiple comparison procedure. This method was performed twice, as described below. The strength of the linear relationship between CYP26 inductions and other variables was assessed with Pearson's product-moment correlation. To see whether certain variables (age and sex) were significantly correlated with CYP26 induction when tRA concentration was controlled for, multiple linear regression modeling was used with the forward stepwise selection procedure. Inclusion into the model was determined by the significance of each variable's partial

correlation coefficient. Age, weight, cumulative dose, and CYP26 mRNA levels of 13-*cis* RA responders and non-responders were compared using the two-sample Student t-test. All data are summarized as means ± SEM. Significance was attained with $P<0.05$ for a two-tailed hypothesis. The data were analyzed with SAS statistical software version 9.1 (SAS Institute, Inc. Cary, NC).

RESULTS

CYP26 enzymatic activity is variable in normal human skin

We first assessed basal (constitutive) CYP26 enzymatic activity in normal human skin (82 males and 36 females). The age range of subjects was 19 to 62 years, with a mean age of 35.9 ± 0.9 years. CYP26 enzymatic activity, as determined by formation of 4-hydroxy RA, ranged from 1 to 180 pg/min per mg total epidermal microsomal protein, with a mean level of 42.7 ± 3.5 pg/min per mg total epidermal microsomal protein (Fig. 1). There was no significant difference ($P=0.29$) in enzymatic activity between females and males (43.8 ± 6.9 and 42.1 ± 4.0 pg/min per mg, respectively). We also found no significant correlation between CYP26 activity and age when comparing all subjects ($r=0.05$; $P=0.59$), only female subjects (age range 20-62, mean age 37.3 ± 1.8; $r=0.25$; $P=0.15$), and only male subjects (age range 19-60, mean age 35.2 ± 1.1; $r=0.06$; $P=0.58$).

CYP26 enzymatic activity is induced in a dose-dependent manner by *t*RA in normal human skin *in vivo*

To investigate the characteristics of CYP26 induction, we applied vehicle and six differing concentrations of *t*RA (0.001%, 0.005%, 0.01%, 0.025%, 0.05% and 0.1%) to buttock skin of healthy subjects (N=29) for 24 hours. No adverse events were experienced by any subject. After obtaining keratome samples of treated and control skin, we measured the inducibility (expression) of CYP26 enzymatic activity. We found a clear dose response, in which subjects expressed higher CYP26 induction with higher concentrations of applied *t*RA. The data were first analyzed in purest statistical form with six individuals treated with five *t*RA concentrations (Fig. 2A). To obtain data from additional patients, a second analysis was performed that included the same cohort of six individuals, in addition to 23 patients that were each exposed to only a single *t*RA concentration, including 0.025% (N=12), 0.05% (N=7), or 0.1% (N=4). Compared with vehicle treatment, both types of analyses revealed significant induction ($P<0.05$) of CYP26 enzymatic activity at every dose, except 0.001% (Fig. 2B). Additionally, using multivariate regression analysis, we did not observe any significant correlation between CYP26 activity and subject age or sex (data not shown).

Isotretinoin responders and non-responders demonstrate similar CYP26 mRNA induction *in vivo*

Acne patients who had been previously treated with one course of 13-*cis* RA were divided into responders and non-responders based on physician interview and retrospective chart review (Table 1). Responders (N=8; 3 males and 5 females; mean age 21.9 ± 2.2 years; age range 16 to 32 years; mean weight 67.5 ± 5.2 kg) were defined as subjects in whom acne clinically resolved after one course of treatment. Non-responders (N=8; 4 males and 4 females; mean age 17.9 ± 1.9 years; range 13 to 30 years; mean weight 61.4 ± 2.7) were defined as patients who did not experience substantial or sustained improvement of acne after a *single* course of 13-*cis* RA, as judged by the physician, patient, or both. There was no significant difference in the mean age ($P=0.19$) or weight ($P=0.32$) of responders versus non-responders at the time of 13-*cis* RA initiation. After completing their initial treatment course, patients were followed by their primary dermatologist or family physician an average of 7.4±1.4 years (range 10 months to 21 years). Cumulative dosage data were available for 7 responders and 7 non-responders. Comparing responders with non-

responders, there was no significant difference (P=0.90) in cumulative dose of 13-*cis* RA (127.8 ± 14.1 mg/kg versus 131.1 ± 20.9 mg/kg, respectively).

In a previous study, we have shown that CYP26 is transcriptionally regulated and that *t*RA application elicits increased CYP26 mRNA synthesis and enzymatic activity.[2] In that study, CYP26 enzymatic activity correlated with mRNA expression. Therefore, we measured CYP26 mRNA (as opposed to enzymatic activity) in the skin of 13-*cis* RA responders and non-responders. The use of punch biopsies, rather than keratome biopsies, in these patients also limited our laboratory assay to mRNA quantification. All subjects were treated with 0.1% *t*RA and vehicle in a paired manner for 24 hours. Side effects occurred in 2 patients (both non-responders) and consisted of mild redness, scaling, and/or itching that readily resolved with emollients or no treatment.

Based on RT-PCR, we found no significant differences (P>0.05) in CYP26 mRNA expression in vehicle-treated skin from responders versus non-responders (data not shown), suggesting that constitutive levels of CYP26 did not differ between the two groups. In *t*RA-treated skin, responders demonstrated a 587±325 fold induction of CYP26 mRNA compared with vehicle-treated skin (Fig. 3). Non-responders demonstrated a 657±227 fold induction compared with vehicle-treated skin. Comparing fold induction between responders and non-responders, there were no statistically significant differences (P=0.84, Fig. 3). Additionally, no statistically significant differences were found when comparing male (N=3) versus female (N=5) responders (P=0.43), or male (N=4) versus female (N=4) non-responders (P=0.35, data not shown).

DISCUSSION

Isotretinoin, or 13-*cis* RA, is the most effective medication for severe acne. Despite its use for decades, the mechanism of this drug is not fully understood. Current evidence suggests that isotretinoin may affect many of the factors involved in acne pathogenesis, such as androgenic activity, sebum production, bacteria, and inflammation. Indeed, 13-*cis* RA has been shown *in vitro* to decrease androgen production.[12] Isotretinoin also decreases sebocyte proliferation and differentiation, and more recently, investigators have found that 13-*cis* RA induces cell cycle arrest and apoptosis in human sebocyte cell lines.[3,13,14] Additionally, neutrophil migration is inhibited by 13-*cis* RA, and this may be one way 13-*cis* RA targets inflammation.[15]

In humans, 13 *cis*-RA elicits many of its effects by first isomerizing to *t*RA, a retinoid with important and diverse effects on skin homeostasis. CYP26, a member of the cytochrome P450 system, is responsible for initiating the process of *t*RA catabolism, regardless of whether *t*RA is endogenous or derived from exogenous sources (e.g., 13-*cis* RA isomerization or topical *t*RA). CYP26 is highly expressed by epidermal keratinocytes in the basal layer of the epidermis, as well as epithelial cells of the eccrine sweat gland and sebaceous gland.[16] In addition to acting principally on *t*RA, CYP26 is also specifically and directly induced by *t*RA, but not other retinoids.[5] Thus, *t*RA levels in the body are regulated by an orchestrated feedback system.

Based on these observations, we postulated that differences in CYP26 activity may explain some of the variable clinical responses to 13-*cis* RA in acne patients. We hypothesized that non-responders might be “fast-metabolizers” who have greater CYP26 expression or inducibility. We hypothesized that this might result in decreased levels of isotretinoin-derived *t*RA, which in turn leads to decreased therapeutic response.

To investigate this possibility, we first measured CYP26 levels in normal skin. We found wide variability in basal CYP26 enzymatic activity. We interpreted this as a reflection of

genetic polymorphism for CYP26; however, it is worthwhile to note that expression and activity of CYP26 is complex and likely affected by environmental and endogenous factors (e.g., dietary vitamin A, medications, or hormone levels).[17] After application of topical *t*RA, we found that CYP26 induction in healthy subjects was dose-dependent, but not correlated with age or sex.

Next, we examined skin samples taken from 13-*cis* RA responders and non-responders. Of note, responders and non-responders did not exhibit significant differences in sex, age, or cumulative dose of the first course of 13-*cis* RA. Based on our biochemical studies, we found that 13-*cis* RA responders and non-responders had similar constitutive levels of CYP26 mRNA expression in vehicle-treated skin. Following topical *t*RA treatment, both groups also had similar levels of CYP26 mRNA induction, although non-responders had a slightly increased, but not statistically significant, mean induction level. Other factors such as gender did not affect CYP26 induction in responders and non-responders. These results suggest that variation in CYP26 basal expression and/or induction may play only a minor role in determining therapeutic response to isotretinoin.

Thus, additional factors may impact the clinical efficacy of oral 13-*cis* RA. For instance, another cytochrome P450 enzyme, known as CYP2S1, may participate in the hydroxylation of *t*RA. Possibly, acne patients may exhibit differences in the expression of this enzyme. Moreover, little is known about the mechanisms which regulate the rate of isomerization of 13-*cis* RA to *t*RA. Indeed, it is unclear whether isomerization is an active, enzymatically mediated process or a spontaneous phenomenon, or both. Adding to the complexity of this is recent evidence that 13-*cis* RA exerts some biological effects without first converting to *t*RA. Isotretinoin and its metabolites, such as 4-oxo-isotretinoin and 4-hydroxy-isotretinoin, appear to directly alter sebaceous gland function in acne patients.[13,18,19] It remains unclear if these effects are mediated by retinoid receptors or a different pathway.

Our study was limited by the small number of acne patients. Furthermore, it is likely that our attempt to classify patients strictly as responders or non-responders represents another limiting factor. Although numerous clinical scales for grading acne severity exist (e.g., the Leeds acne grading system), these are subjective. Objective or quantitative methods of evaluation, analogous to Breslow depth for melanoma, are still lacking for acne. In this study, we classified subjects as responders or non-responders based on patient history and retrospective chart review. However, in clinical practice response to therapy often depends on the subjective perception of acne severity as judged by individual physicians and patients. Strict criteria for determining which patients necessitate a second course of 13-*cis* RA do not exist.

Overall, a great deal of progress has been made in investigating the mechanisms of isotretinoin in acne, but comparatively much less is known about what governs therapeutic response to this drug. This translational study is one of the first attempting to link biochemical factors (i.e., CYP26 activity) to 13-*cis* RA response. Of note, oral 13-*cis* RA has a number of serious side effects, such as teratogenicity, increased liver enzymes, hypercholesterolemia, and hypertriglyceridemia, as well as less severe effects, such as mucocutaneous dryness and alopecia.[20] Additionally, 13-*cis* RA has been linked to suicidal ideation, although prospective, randomized data which clearly substantiate this remain elusive. Therefore, anticipating which acne patients may require more than one course of 13-*cis* RA for therapeutic response could help practitioners consider other treatment options. In this retrospective study, we attempted to determine whether CYP26 induction could be used as a predictive factor for this purpose. Our data suggest that future studies correlating basal (pre-therapy) CYP26 expression to 13-*cis* RA response in acne patients, as well as studies comparing CYP26 expression in normal versus acne patients, may be more informative in

this regard. Clearly, further research is necessary to determine the mechanisms that are involved in treatment response to isotretinoin.

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ABBREVIATIONS

CYP26	Retinoic acid 4-hydroxylase
***t*RA**	All-*trans* retinoic acid
13-*cis* RA	13-*cis* retinoic acid (isotretinoin)
RAR	Retinoic acid receptor
RXR	Retinoid X receptor
RARE	Retinoic acid response element

REFERENCES

1. Fisher GJ, Voorhees JJ. Molecular mechanisms of retinoid actions in skin. Faseb J. 1996; 10:1002–13. [PubMed: 8801161]
2. Marikar Y, Wang Z, Duell EA, Petkovich M, Voorhees JJ, Fisher GJ. Retinoic acid receptors regulate expression of retinoic acid 4-hydroxylase that specifically inactivates all-trans retinoic acid in human keratinocyte HaCaT cells. J Invest Dermatol. 1998; 111:434–9. [PubMed: 9740237]
3. Tsukada M, Schroder M, Roos TC, Chandraratna RA, Reichert U, Merk HF, et al. 13-cis retinoic acid exerts its specific activity on human sebocytes through selective intracellular isomerization to all-trans retinoic acid and binding to retinoid acid receptors. J Invest Dermatol. 2000; 115:321–7. [PubMed: 10951254]
4. Petkovich PM. Retinoic acid metabolism. J Am Acad Dermatol. 2001; 45:S136–42. [PubMed: 11606945]
5. Duell EA, Kang S, Voorhees JJ. Retinoic acid isomers applied to human skin in vivo each induce a 4-hydroxylase that inactivates only trans retinoic acid. J Invest Dermatol. 1996; 106:316–20. [PubMed: 8601734]
6. Lehucher-Ceyrac D, Weber-Buisset MJ. Isotretinoin and acne in practice: a prospective analysis of 188 cases over 9 years. Dermatology. 1993; 186:123–8. [PubMed: 8428040]
7. Layton AM, Cunliffe WJ. Guidelines for optimal use of isotretinoin in acne. J Am Acad Dermatol. 1992; 27:S2–7. [PubMed: 1460120]
8. Leyden JJ. Oral isotretinoin. How can we treat difficult acne patients? Dermatology. 1997; 195(Suppl 1):29–33. discussion 8-40. [PubMed: 9310743]
9. Lee SJ, Perera L, Coulter SJ, Mohrenweiser HW, Jetten A, Goldstein JA. The discovery of new coding alleles of human CYP26A1 that are potentially defective in the metabolism of all-trans retinoic acid and their assessment in a recombinant cDNA expression system. Pharmacogenet Genomics. 2007; 17:169–80. [PubMed: 17460545]
10. Duell EA, Astrom A, Griffiths CE, Chambon P, Voorhees JJ. Human skin levels of retinoic acid and cytochrome P-450-derived 4-hydroxyretinoic acid after topical application of retinoic acid in vivo compared to concentrations required to stimulate retinoic acid receptor-mediated transcription in vitro. J Clin Invest. 1992; 90:1269–74. [PubMed: 1328295]

11. Kang S, Cho S, Chung JH, Hammerberg C, Fisher GJ, Voorhees JJ. Inflammation and extracellular matrix degradation mediated by activated transcription factors nuclear factor-kappaB and activator protein-1 in inflammatory acne lesions in vivo. Am J Pathol. 2005; 166:1691–9. [PubMed: 15920154]
12. Karlsson T, Vahlquist A, Kedishvili N, Torma H. 13-cis-retinoic acid competitively inhibits 3 alpha-hydroxysteroid oxidation by retinol dehydrogenase RoDH-4: a mechanism for its anti-androgenic effects in sebaceous glands? Biochem Biophys Res Commun. 2003; 303:273–8. [PubMed: 12646198]
13. Nelson AM, Gilliland KL, Cong Z, Thiboutot DM. 13-cis Retinoic acid induces apoptosis and cell cycle arrest in human SEB-1 sebocytes. J Invest Dermatol. 2006; 126:2178–89. [PubMed: 16575387]
14. Nelson AM, Zhao W, Gilliland KL, Zaenglein AL, Liu W, Thiboutot DM. Neutrophil gelatinase-associated lipocalin mediates 13-cis retinoic acid-induced apoptosis of human sebaceous gland cells. J Clin Invest. 2008; 118:1468–78. [PubMed: 18317594]
15. Wozel G, Chang A, Zultak M, Czarnetzki BM, Happle R, Barth J, et al. The effect of topical retinoids on the leukotriene-B4-induced migration of polymorphonuclear leukocytes into human skin. Arch Dermatol Res. 1991; 283:158–61. [PubMed: 1651071]
16. Heise R, Mey J, Neis MM, Marquardt Y, Joussen S, Ott H, et al. Skin retinoid concentrations are modulated by CYP26AI expression restricted to basal keratinocytes in normal human skin and differentiated 3D skin models. J Invest Dermatol. 2006; 126:2473–80. [PubMed: 16778795]
17. Ross AC. Retinoid production and catabolism: role of diet in regulating retinol esterification and retinoic Acid oxidation. J Nutr. 2003; 133:291S–6S. [PubMed: 12514312]
18. Zouboulis CC. Isotretinoin revisited: pluripotent effects on human sebaceous gland cells. J Invest Dermatol. 2006; 126:2154–6. [PubMed: 16983322]
19. Baron JM, Heise R, Blaner WS, Neis M, Joussen S, Dreuw A, et al. Retinoic acid and its 4-oxo metabolites are functionally active in human skin cells in vitro. J Invest Dermatol. 2005; 125:143–53. [PubMed: 15982314]
20. Ellis CN, Krach KJ. Uses and complications of isotretinoin therapy. J Am Acad Dermatol. 2001; 45:S150–7. [PubMed: 11606947]

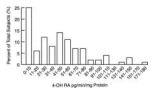

Figure 1.
Basal CYP26 enzymatic activity in normal human skin. After isolating microsomal fractions from the epidermis of keratome biopsies obtained from healthy subjects (N=118), basal CYP26 enzymatic activity (expressed as formation of 4-hydroxy RA [pg/min] per mg of total isolated protein) was determined.

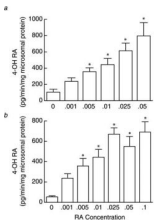

Figure 2.

Dose dependence of CYP26 enzymatic activity with tretinoin concentration. Following topical treatment of normal human skin with tretinoin (*t*RA) for 24 hours, keratome biopsies were obtained. After isolating microsomal fractions from the epidermis, CYP26 enzymatic activity was determined based on formation of 4-hydroxy RA [pg/min] per mg of total isolated protein. (a) Induction of CYP26 enzyme in six individuals treated with vehicle and the indicated concentrations of *t*RA is shown. (b) This panel displays data obtained from the same cohort as in "a" plus 23 patients that were treated with vehicle and only a single concentration of *t*RA, including 0.025% (N=12), 0.05% (N=7), or 0.1% (N=4). Fold changes are expressed as means + SEM, relative to untreated skin (normalized to 1, not shown). *P<0.05, compared with vehicle-treated skin.

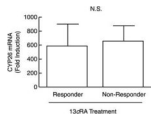

Figure 3.

CYP26 mRNA induction in isotretinoin responders and non-responders. Following topical application of 0.1% *t*RA and vehicle under occlusion for 24 hours, punch biopsies were taken from acne patients who had previously responded (N=8) or did not respond (N=8) to one course of isotretinoin. Total RNA was extracted from these biopsies, and CYP26 mRNA was quantified by real-time polymerase chain reaction. Fold changes are expressed as means + SEM, relative to untreated skin (normalized to 1, not shown). NS, not significant (P>0.05)

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TABLE 1

Clinical information for acne patients who responded or did not respond to one course of isotretinon.

Responder	Age at Drug Initiation	Gender	Ethnicity	Weight (kg)	Cumulative Dose (mg/kg)
1	25	female	Caucasian	61.2	117.6
2	16	male	Caucasian	68	52.9
3	32	female	Caucasian	54.5	157.9
4	17	male	Caucasian	61.3	132.1
5	18	female	Caucasian	56.7	141.1
6	29	female	Caucasian	77.1	125.6
7	19	female	Caucasian	68	167.6
8	18	male	Caucasian	97.7	not available
Mean±SE	**21.9±2.2**			**67.5±5.2**	**127.8±14.1**

Non-Responder	Age at Drug Initiation	Gender	Ethnicity	Weight (kg)	Cumulative Dose (mg/kg)
1	16	male	Caucasian	65	166.15
2	19	male	Black	74.8	88.2
3	19	female	Caucasian	54.5	231
4	16	male	Caucasian	63.5	not available
5	14	female	Caucasian	49.9	136.3
6	30	male	Caucasian	61.2	116.2
7	16	female	Caucasian	56.7	84.6
8	13	female	Asian/Caucasian	65.8	95.4
Mean±SE	**17.9±1.9**			**61.4±2.7**	**131.12±20.9**