Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 303 (2003) 273–278
BBRC
www.elsevier.com/locate/ybbrc

13-*cis*-Retinoic acid competitively inhibits 3α-hydroxysteroid oxidation by retinol dehydrogenase RoDH-4: a mechanism for its anti-androgenic effects in sebaceous glands?

Teresa Karlsson,[a] Anders Vahlquist,[a] Natalia Kedishvili,[b] and Hans Törmä[a,*]

[a] *Department of Medical Sciences/Dermatology and Venereology, University Hospital, Uppsala, Sweden*
[b] *Division of Molecular Biology and Biochemistry, University of Missouri, Kansas City, MO, USA*

Received 11 February 2003

Abstract

Retinol dehydrogenase-4 (RoDH-4) converts retinol and 13-*cis*-retinol to corresponding aldehydes in human liver and skin in the presence of NAD^+. RoDH-4 also converts 3α-androstanediol and androsterone into dihydrotestosterone and androstanedione, which may stimulate sebum secretion. This oxidative 3α-hydroxysteroid dehydrogenase (3α-HSD) activity of RoDH-4 is competitively inhibited by retinol and 13-*cis*-retinol. Here, we further examine the substrate specificity of RoDH-4 and the inhibition of its 3α-HSD activity by retinoids. Recombinant RoDH-4 oxidized 3,4-didehydroretinol—a major form of vitamin A in the skin—to its corresponding aldehyde. 13-*cis*-retinoic acid (isotretinoin), 3,4-didehydroretinoic acid, and 3,4-didehydroretinol, but not all-*trans*-retinoic acid or the synthetic retinoids acitretin and adapalene, were potent competitive inhibitors of the oxidative 3α-HSD activity of RoDH-4, i.e., reduced the formation of dihydrotestosterone and androstandione in vitro. Extrapolated to the in vivo situation, this effect might explain the unique sebosuppressive effect of isotretinoin when treating acne.

Keywords: Acne; Isotretinoin; Retinol dehydrogenase; Skin; Epidermis; Retinoid; Enzyme kinetics; Androgen; Retinoic acid

Retinoids (natural and synthetic derivatives of vitamin A) are known to affect the growth and differentiation of many types of cells. Retinoids regulate gene transcription by binding to and activating retinoic acid receptors (RAR-α, -β, and -γ) and 9-*cis*-retinoic acid (9cRA) receptors (RXR-α, -β, and -γ). All-*trans*-retinoic acid, atRA, is generally thought to exert most biological actions of retinoids. Retinol is metabolized to atRA in a tightly controlled two-step oxidative process, starting with the conversion of all-*trans*-retinol to all-*trans*-retinaldehyde, followed by the conversion of all-*trans*-retinaldehyde to atRA (for a review, see [1] or [2] and references therein). Oxidoreductases known as retinol dehydrogenases (RDHs) catalyze the first reaction, which appears to be rate limiting [2] while cytosolic retinaldehyde dehydrogenases are thought to catalyze the second reaction [3,4].

Several isoenzymes belonging to the short chain dehydrogenase/reductase (SDR) superfamily with various co-factor and substrate preferences have been cloned from mammals. Some of these only recognize all-*trans*-retinol as substrate [5–8], whereas others may also metabolize *cis*-retinols (9-, 11-, or 13-*cis*-retinol) [9–16]. Many of these SDRs also oxidize androgens, primarily the 3α-hydroxysteroids 3α-adiol (5α-androstan-3α,17β-diol) and androsterone (5α-androstan-3α-ol-17-one) into DHT (dihydrotestosterone) and androstanedione (5α-androstan-3,17-dione), respectively [2]. Recently, the first human counterpart to a RDH-like SDR, called RoDH-4, was cloned [17]. RoDH-4, a NAD^+-preferring oxidoreductase expressed in the liver, is able to oxidize all-*trans*-retinol and 13-*cis*-retinol as well as 3α-adiol and androsterone, thus being the first *human* microsomal enzyme capable of metabolizing all-*trans*-retinol to its corresponding aldehyde. Interestingly, the oxidative 3α-HSD activity of RoDH-4 is competitively inhibited by both all-*trans*-retinol and 13-*cis*-retinol.

* Corresponding author. Fax: +46-18-611-26-80.
E-mail address: Hans.Torma@medsci.uu.se (H. Törmä).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00332-2

More recently, RoDH-4 has also been reported in human epidermis under the name hRDH-E [18]. This is of particular interest since vitamins in the form of all-*trans*- and 3,4-didehydroretinol are metabolized to retinoic acids in the skin [19,20], and androgens, such as DHT, are potent stimulators of sebocyte growth and differentiation [21,22]. For years, skin disorders such as acne and psoriasis have been treated with natural (atRA and 13-*cis*-retinoic acid, 13cRA) or synthetic (e.g., acitretin and adapalene) retinoids either topically or orally, but their exact mechanism of action in many cases remains an enigma. To shed further light on the complex interactions between retinoid therapy and the metabolism of vitamin A and androgens in the skin, we herein examine the in vitro enzymatic activity of RoDH-4 in terms of retinoid substrate specificity and inhibition of androgen metabolism by different retinoids.

Materials and methods

Expression of RoDH-4 in Sf9 cells and preparation of microsomal fractions. Sf9 cells (Invitrogen, Carlsbad, CA) were cultured and transfected with the RoDH-4-pVL1392 expression vector as previously described [17]. After three days of incubation, cells were collected and homogenized in 0.01 M potassium phosphate, pH 7.4, 0.25 M sucrose, 0.1 mM EDTA, and 0.1 mM DTT. Microsomes were pelleted by centrifugation, suspended, and stored as previously described [17].

Enzymatic analysis of retinol dehydrogenase activity by HPLC analysis. Assay of RoDH-4-catalyzed oxidation of retinol and 3,4-didehydroretinol was performed by the procedure described by Gough et al. [17]. In brief, RoDH-4-containing microsomes were incubated with 10 μM retinol and 1 mM co-factor (NAD^+) in 90 mM potassium phosphate, pH 7.3, and 40 mM KCl at 37 °C in glass tubes for 30 min, and then extracted with hexane. After evaporation under N_2, retinoids were dissolved in methanol and analyzed by HPLC.

Reversed-phase chromatography was performed on a Nucleosil 5 μm PEAB-ODS column (3.6 × 150 mm) eluted with acetonitrile:water (85:15). The HPLC equipment is described in detail elsewhere [23].

Retinoid inhibition of 3α-HSD activity. Inhibition assays of androsterone/3α-adiol oxidation were performed as previously described [17]. Initial inhibition reactions were performed in 90 mM potassium phosphate, pH 7.3, and 40 mM KCl at 37 °C in siliconized glass tubes, with 1 mM NAD^+, 0.125 μM ^{3}H-labeled steroid (NEN Life Science Products, Boston, MA) and 10 μM of each retinoid (Sigma–Aldrich, St. Louis, MO). For determination of inhibition constants (K_i), four concentrations of steroid and three concentrations of retinoid were used. Aqueous solutions of ^{3}H-labeled androsterone and 3α-adiol were prepared from 100× radiolabeled stock in dimethyl sulfoxide, adding equimolar amounts of bovine serum albumin to improve solubility followed by sonicating for 10 min. Retinoids were added from 100× stock solutions in either ethanol [atRA (tretinoin), 9cRA, 13cRA (isotretinoin/Accutane), 3,4-didehydroretinoic acid (ddRA), and 3,4-didehydroretinol] or dimethyl sulfoxide [acitretin (Neotigason), CD271 (adapalene/Differin), and CD367], so that the final concentration of organic solvent was always 1%. Control reactions always contained 1% of the corresponding solvent. The 250-μl reactions were started by adding RoDH-4-containing microsomes and stopped after 15 min by the addition of 3 ml methylene chloride. All retinoid solutions and reaction mixtures were kept in the dark. After removing the aqueous phase and evaporating under N_2, steroids were dissolved in 50 μl methylene chloride, spotted onto aluminum oxide thin layer chromatography plates (Sigma–Aldrich), and resolved by development in chloroform/ethyl acetate (3:1). The lanes were cut into 1-cm pieces and subjected to liquid scintillation counting. For kinetic evaluation, each data set was evaluated for fit to different types of inhibition using the GraFit software (Erithacus Software, Horley, UK).

Results

Functional analysis of RoDH-4: 3,4-didehydroretinol is also a substrate of RoDH-4

Our initial experiment confirmed that microsomes from Sf9 cells transfected with the RoDH-4 expression vector oxidized all-*trans*-retinol to all-*trans*-retinal in the presence of NAD^+ (Fig. 1B), whereas in the absence of cofactor, the activity was in the same range as control microsomes (Fig. 1A). Endogenous microsomal activity of the Sf9 cells probably explains the background activity of the controls. When microsomes were incubated with 3,4-didehydroretinol and NAD^+, 3,4-didehydroretinal was produced (Fig. 1D), but this process was only 60% as efficient compared to when retinol was used as substrate. In the absence of NAD^+ there was only minimal formation of 3,4-didehydroretinal (Fig. 1C).

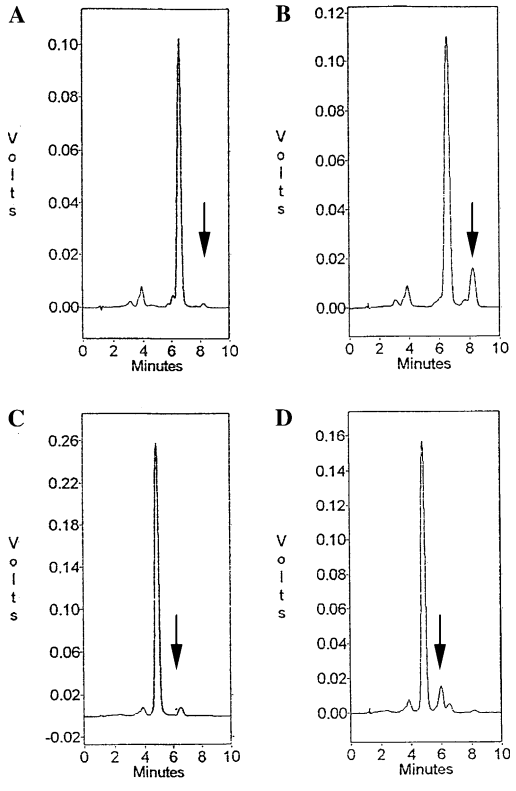

Fig. 1. RoDH-4 oxidizes all-*trans*- and 3,4-didehydroretinol to aldehydes in the presence of NAD^+. HPLC chromatograms show the oxidation of 10 μM all-*trans*-(A,B) and 3,4-didehydroretinol (C,D) in the absence (A,C) and presence (B,D) of 1 mM NAD^+. Arrows indicate the elution time of authentic aldehyde products.

Retinoids inhibit the androgen oxidizing activity of RoDH-4

All-*trans*- and 13-*cis*-retinol have previously been shown to competitively inhibit the oxidation of androsterone by RoDH-4 [17]. Since our initial results identified 3,4-didehydroretinol as a new substrate for RoDH-4, we included this substance when assessing the ability of various retinoic acids (atRA, 9cRA, 13cRA, and ddRA) to inhibit the androgen oxidizing activity of RoDH-4 in vitro.

Similar results were obtained using either androsterone or 3α-adiol as substrate (Fig. 2). At 0.125 μM of steroid substrate (approx. K_m value), 10 μM of 13cRA, ddRA, and 3,4-didehydroretinol were the most efficient inhibitors, reducing the RoDH-4 activity to about 20%, 35%, and 23% of control, respectively. atRA, 9cRA, and acitretin had no significant effects in this system, whereas the two synthetic retinoids CD271 and CD367 inhibited androsterone oxidation to 70% and 3α-adiol oxidation to about 50% of control values.

To further characterize the inhibitory effects of 13cRA, ddRA, and 3,4-didehydroretinol on steroid oxidation, we performed kinetic studies. All three retinoids acted as competitive inhibitors of the oxidation of androsterone and 3α-adiol (see Fig. 3 for representative graphs) with apparent K_i values, indicating the following order of inhibition potency: 13cRA > 3,4-didehydroretinol > ddRA (Table 1).

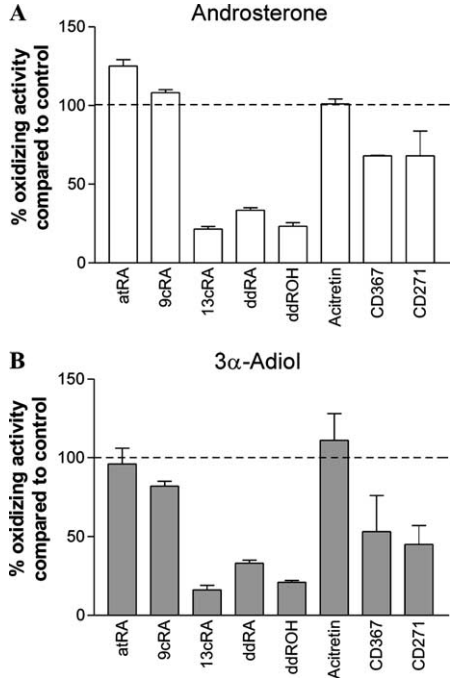

Fig. 2. Androgen oxidation by RoDH-4 is inhibited by 13-*cis*-3,4-didehydroretinoic acid, 3,4-didehydroretinol, CD271, and CD367 but not acitretin, all-*trans*-, or 9-*cis*-retinoic acid. Conversion of androsterone to androstanedione (A) and 3α-adiol to dihydrotestosterone (B) in the presence of 10 μM of various retinoids. Both androgens were added at a concentration of 0.125 μM. The results are shown as percent of product formation of controls (with no retinoid added) and represent mean values of duplicate experiments ± SD.

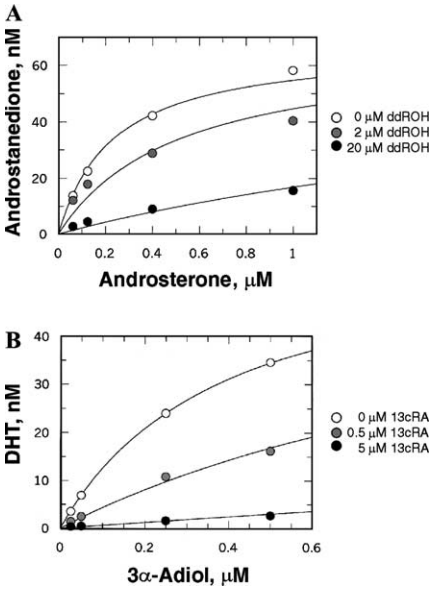

Fig. 3. Competitive inhibition of RoDH-4-mediated androgen oxidation by retinoids. Kinetic studies of inhibition of androsterone oxidation by ddROH (A) and of 3α-adiol oxidation by 13cRA (B) were performed as described under Material and methods. Androsterone was used at concentrations 0.0625, 0.125, 0.5, and 1 μM in combination with 0, 2, or 20 μM ddROH (A). 3α-Adiol was used at concentrations 0.025, 0.05, 0.25, and 0.5 μM with 0, 0.5, or 5 μM 13cRA (B).

Table 1
Inhibitory constants (K_i)[a] for various retinoids on the oxidation of androsterone and 3α-adiol by RoDH-4

	Substrate	
Inhibitor[b]	Androsterone[c] K_i (μM)	3α-Adiol[d] K_i (μM)
13-*cis*-Retinoic acid (13cRA)	1.1 ± 0.3	0.21 ± 0.01
3,4-Didehydroretinoic acid (ddRA)	3.5 ± 1.1	6.9 ± 2.7
3,4-Didehydroretinol	2.5 ± 0.6	2.2 ± 0.2

[a] Presented as means ± SEM of two or three experiments.
[b] Each inhibitor was used at three concentrations (0–20 μM).
[c] Concentrations 0.0625, 0.125, 0.5, and 1 μM were used.
[d] Concentrations 0.025, 0.05, 0.25, and 0.5 μM were used.

Discussion

In the present report, we show that recombinant RoDH-4 expressed in insect cells converts 3,4-didehydroretinol to its corresponding aldehyde in the presence of NAD^+. To the best of our knowledge, only one previous report has addressed a similar matter, viz. that

rat RoDH-1 recognizes 3,4-didehydroretinol in the free form, but not when bound to cellular retinol-binding protein, as substrate for oxidation [24]. Human epidermis is the only mammalian tissue known to contain significant amounts of the 3,4-didehydroretinoids (about 30% of the total retinoid content) [25], but information regarding their function as well as metabolic processing in the skin is scarce. RoDH-4 is an attractive candidate enzyme in this context, controlling not only the epidermal generation of retinal and atRA [18], but possibly also of 3,4-didehydroretinal (this study), leading to the formation of ddRA with similar, albeit not identical, biological effects as atRA ([26] and see below).

Another aspect of this study concerns the metabolic interactions between retinoids and 3α-hydroxysteroids at the level of microsomal retinoldehydrogenase/3α-HSD activity. It was previously shown that all-*trans*- and 13-*cis*-retinol competitively inhibit the RoDH-4 catalyzed oxidation of androsterone with K_i values of 5.8 and 3.5 μM, respectively [17]. This is not unexpected, since all these compounds are substrates for oxidation. Our present results show that 13cRA is an even more potent competitive inhibitor of the oxidative 3α-HSD activity of RoDH-4 in vitro, with apparent K_i-values of 1.1 and 0.21 μM for androsterone and 3α-adiol as substrate, respectively. Biswas and Russell [27] have previously shown that the conversion of 3α-adiol to DHT by a "human RoDH" cloned from prostate was inhibited by 13cRA, albeit less efficiently ($K_i = 30\,\mu M$) than in our study of RoDH-4. Furthermore, 13cRA is a potent inhibitor of human, mouse, and bovine 9-*cis*/11-*cis*-RoDHs [28], and although the substrates used in these studies were 9-*cis*- and 11-*cis*-retinol and not androgens, we believe that the results further reinforce the efficiency of 13cRA as an inhibitor of RoDHs.

Interestingly, 13cRA (isotretinoin, Roaccutane/Accutane) is widely used as oral treatment of severe acne (reviewed in [29]), a disease where excessive production of active androgens (primarily DHT) in the skin and hence overstimulation of the sebaceous gland has been incriminated [30]. 13cRA reduces the size and sebum secretion of the sebaceous glands more potently than any other known retinoid, but its mode of action is an enigma since it does not bind to the RAR/RXRs. Recently, it was proposed that isomerization of 13cRA to atRA in sebocytes explains its biological effect [31]. However, atRA, which binds to the RARs, is a less effective anti-acne agent than 13cRA, suggesting a different mechanism of action. Since 13-*cis*-RA is readily detected in the sebaceous glands during therapy [32], we hypothesize that a major effect of this drug in acne is to competitively inhibit RoDH-4 in the sebocytes, thus lowering the production of DHT from 3α-adiol (see Fig. 4) and reverting the androgen-induced hyperactivity of sebaceous glands. In support of this theory the K_i value for 13cRA inhibition of 3α-adiol oxidation (0.21 μM) was found to be well below the serum concentration of 13cRA attained during acne treatment [33–35].

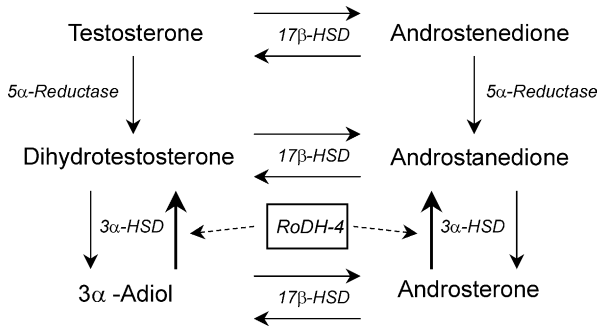

Fig. 4. Proposed role of RoDH-4 in androgen metabolism and the influence of inhibitory retinoids. 5α-Reductase converts testosterone and androstenedione irreversibly into dihydrotestosterone (DHT) and androstanedione, respectively. DHT is metabolized to 3α-adiol by reductive 3α-HSDs, thereby becoming inactivated. By 17β-HSD activity, 3α-adiol undergoes oxidation into androsterone, which is eliminated after glucuronidation. The oxidative 3α-HSD function of RoDH-4 (bold arrows) may regenerate DHT by (1) oxidizing 3α-adiol into DHT and (2) oxidizing androsterone into androstanedione, which in turn may undergo reduction into DHT by a presently unknown 17β-HSD. The negative signs indicate where 13cRA (isotretinoin), 3,4-didehydroretinol, and ddRA exert their inhibitory effect.

Two of the other natural retinoids, 3,4-didehydroretinol and ddRA, were also strong inhibitors of the androgen oxidizing activity of RoDH-4, with apparent K_i-values of 2.5 and 3.5 μM (androsterone oxidation) and 2.2 and 6.9 μM (3α-adiol oxidation), respectively. This is especially interesting since ddRA is almost as effective as 13cRA in two animal models of acne; the rhino mouse utricle assay and the hamster ear sebaceous gland assay [36]. In contrast, atRA and 9cRA, which have no sebosuppressive effects in vivo [37], did not inhibit the oxidative 3α-HSD activity.

Three synthetic retinoids—acitretin, CD271 (adapalene), and CD367—were included in our study because they are not substrates for or products of RoDH-mediated oxidation. While acitretin had no effect, CD271 and CD367 reduced the oxidative 3α-HSD activity of RoDH-4 by 30–60% (Fig. 2). This may not represent a truly competitive inhibition since the androsterone oxidizing activity remained at about 40% or more even when higher concentrations (>10 μM) of CD271 and CD367 were used (not shown).

In summary, the present study shows that 13cRA (isotretinoin) inhibits the 3α-HSD activity of RoDH-4 in vitro. If this inhibition is important for the anti-androgenic effects of 13cRA in vivo remains to be studied.

Acknowledgments

This work was supported by grants from the The Swedish Research Council (71x-07133) and the Edvard Welander and Finsen foundations. We thank S.V. Chetyrkin for expert technical help in performing

and evaluating the kinetic studies. Dr. Serge Michel (Galderma R&D, Sophia-Antipolis, France) generously supplied CD271 and CD367.

References

[1] G. Duester, Families of retinoid dehydrogenases regulating vitamin A function: production of visual pigment and retinoic acid, Eur. J. Biochem. 267 (2000) 4315–4324.
[2] J.L. Napoli, Interactions of retinoid binding proteins and enzymes in retinoid metabolism, Biochim. Biophys. Acta 1440 (1999) 139–162.
[3] X. Wang, P. Penzes, J.L. Napoli, Cloning of a cDNA encoding an aldehyde dehydrogenase and its expression in *Escherichia coli*. Recognition of retinal as substrate, J. Biol. Chem. 271 (1996) 16288–16293.
[4] Z. El Akawi, J.L. Napoli, Rat liver cytosolic retinal dehydrogenase: Comparison of 13-*cis*-, 9-*cis*-, and all-*trans*-retinal as substrates and effects of cellular retinoid-binding proteins retinoic acid on activity, Biochemistry 33 (1994) 1938–1943.
[5] X. Chai, Y. Zhai, J.L. Napoli, Cloning of a rat cDNA encoding retinol dehydrogenase isozyme type III, Gene 169 (1996) 219–222.
[6] X.Y. Chai, M.H.E.M. Boerman, Y. Zhai, J.L. Napoli, Cloning of a cDNA for liver microsomal retinol dehydrogenase—a tissue-specific, short-chain alcohol dehydrogenase, J. Biol. Chem. 270 (1995) 3900–3904.
[7] X.Y. Chai, Y. Zhai, G. Popescu, J.L. Napoli, Cloning of a cDNA for a second retinol dehydrogenase type II—expression of its mRNA relative to type I, J. Biol. Chem. 270 (1995) 28408–28412.
[8] F. Haeseleer, J. Huang, L. Lebioda, J.C. Saari, K. Palczewski, Molecular characterization of a novel short-chain dehydrogenase/reductase that reduces all-*trans*-retinal, J. Biol. Chem. 273 (1998) 21790–21799.
[9] C.A. Driessen, H.J. Winkens, E.D. Kuhlmann, A.P. Janssen, A.H. van Vugt, A.F. Deutman, J.J. Janssen, The visual cycle retinol dehydrogenase: possible involvement in the 9-*cis* retinoic acid biosynthetic pathway, FEBS Lett. 428 (1998) 135–140.
[10] C.A. Driessen, B.P.M. Janssen, H.J. Winkens, A.H.M. Vanvugt, T.L.M. Deleeuw, J.J.M. Janssen, Cloning and expression of a cDNA encoding bovine retinal pigment epithelial 11-*cis* retinol dehydrogenase, Invest. Ophth. Vis. Sci. 36 (1995) 1988–1996.
[11] J. Mertz, E. Shang, R. Piantedosi, S. Wei, D. Wolgemuth, W. Blaner, Identification and characterization of a stereospecific human enzyme that catalyzes 9-*cis*-retinol formation, J. Biol. Chem. 272 (1997) 11744–11749.
[12] A. Simon, U. Hellman, C. Wernstedt, U. Eriksson, The retinal pigment epithelial-specific 11-*cis* retinol dehydrogenase belongs to the family of short chain alcohol dehydrogenases, J. Biol. Chem. 270 (1995) 1107–1112.
[13] A. Simon, J. Lagercrantz, S. Bajalica-Lagercrantz, U. Eriksson, Primary structure of human 11-*cis* retinol dehydrogenase and organization and chromosomal localization of the corresponding gene, Genomics 36 (1996) 424–430.
[14] X. Chai, Y. Zhai, J.L. Napoli, cDNA cloning and characterization of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase, J. Biol. Chem. 272 (1997) 33125–33131.
[15] J. Su, X. Chai, B. Kahn, J.L. Napoli, cDNA cloning, tissue distribution, and substrate characteristics of a *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase isozyme, J. Biol. Chem. 273 (1998) 17910–17916.
[16] J. Wang, X. Chai, U. Eriksson, J.L. Napoli, Activity of human 11-*cis*-retinol dehydrogenase (Rdh5) with steroids and retinoids and expression of its mRNA in extra-ocular human tissue, Biochem. J. 338 (1999) 23–27.
[17] W.H. Gough, S. Van Ooteghem, T. Sint, N.Y. Kedishvili, cDNA cloning and characterization of a new human microsomal NAD^+-dependent dehydrogenase that oxidizes all-*trans*-retinol and 3α-hydroxysteroids, J. Biol. Chem. 273 (1998) 19778–19785.
[18] V. Jurukovski, N.G. Markova, N. Karaman-Jurukovska, R.K. Randolph, J. Su, J.L. Napoli, M. Simon, Cloning and characterization of retinol dehydrogenase transcripts expressed in human epidermal keratinocytes, Mol. Genet. Metabol. 67 (1999) 62–73.
[19] R.K. Randolph, M. Simon, Characterization of retinol metabolism in cultured human epidermal keratinocytes, J. Biol. Chem. 268 (1993) 9198–9205.
[20] H. Törmä, O. Rollman, A. Vahlquist, The vitamin A metabolism and expression of retinoid-binding proteins differ in HaCaT cells and normal human keratinocytes, Arch. Dermatol. Res. 291 (1999) 339–345.
[21] H. Akamatsu, C.C. Zouboulis, C.E. Orfanos, Control of human sebocyte proliferation in vitro by testosterone and 5-α-dihydrotestosterone is dependent on the localization of the sebaceous glands, J. Invest. Dermatol. 99 (1992) 509–511.
[22] W. Chen, D. Thiboutot, C.C. Zouboulis, Cutaneous androgen metabolism: basic research and clinical perspectives, J. Invest. Dermatol. 119 (2002) 992–1007.
[23] A. Vahlquist, H. Törmä, O. Rollman, E. Andersson, High-performance liquid chromatography of natural and synthetic retinoids in human skin samples, in: L. Packer (Ed.), Methods Enzymol., Retinoids Part B, vol. 190, Academic Press, Inc, San Diego, 1990, pp. 163–174.
[24] M.H. Boerman, J.L. Napoli, Characterization of a microsomal retinol dehydrogenase: a short-chain alcohol dehydrogenase with integral and peripheral membrane forms that interacts with holo-CRBP (type I), Biochemistry 34 (1995) 7027–7037.
[25] A. Vahlquist, J.B. Lee, G. Michaëlsson, O. Rollman, Vitamin A in human skin: II. Concentrations of carotene, retinol and dehydroretinol in various components of normal skin, J. Invest. Dermatol. 79 (1982) 94–97.
[26] H. Törmä, D. Asselineau, E. Andersson, B. Martin, P. Reiniche, P. Chambon, B. Shroot, M. Darmon, A. Vahlquist, Biological activities of retinoic acid and 3,4-didehydroretiacid acid are similar and correlate to receptor affinities and transactivation properties, J. Invest. Dermatol. 102 (1994) 49–54.
[27] M.G. Biswas, D.W. Russell, Expression cloning and characterization of oxidative 17β- and 3α-hydroxysteroid dehydrogenases from rat and human prostate, J. Biol. Chem. 272 (1997) 15959–15966.
[28] M.V. Gamble, N.L. Mata, A.T. Tsin, J.R. Mertz, W.S. Blaner, Substrate specificities and 13-*cis*-retinoic acid inhibition of human, mouse and bovine *cis*-retinol dehydrogenases, Biochim. Biophys. Acta 1476 (2000) 3–8.
[29] C.E. Orfanos, C.C. Zouboulis, Oral retinoids in the treatment of seborrhoea and acne, Dermatology 196 (1998) 140–147.
[30] W.J. Cunliffe, The sebaceous gland and acne—40 years on, Dermatology 196 (1998) 9–15.
[31] M. Tsukada, M. Schroder, T.C. Roos, R.A. Chandraratna, U. Reichert, H.F. Merk, C.E. Orfanos, C.C. Zouboulis, 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. 115 (2000) 321–327.
[32] A. Vahlquist, O. Rollman, D.B. Holland, W.J. Cunliffe, Isotretinoin treatment of severe acne affects the endogenous concentration of vitamin A in sebaceous glands, J. Invest. Dermatol. 94 (1990) 496–498.
[33] O. Rollman, A. Vahlquist, Oral isotretinoin (13-*cis*-retinoic acid) therapy in severe acne: drug and vitamin A concentrations in serum and skin, J. Invest. Dermatol. 86 (1986) 384–389.
[34] G.A. Fex, A. Aronsson, A. Andersson, K. Larsson, P. Nilsson-Ehle, In vivo effects of 13-*cis* retinoic acid treatment on the concentration of proteins and lipids in serum, Eur. J. Clin. Chem. Clin. Biochem. 34 (1996) 3–7.

[35] R.K. Brazzell, F.M. Vane, C.W. Ehmann, W.A. Colburn, Pharmacokinetics of isotretinoin during repetitive dosing to patients, Eur. J. Clin. Pharmacol. 24 (1983) 695–702.
[36] S. Shapiro, G. Bryce, T. Doran, J. McLane, Pharmacology of retinoids in models of photodamage and acne, in: M. Livrea, L. Packer (Eds.), vol. Retinoids. Progress in research and clinical applications, Marcel Dekker Inc, New York, 1993, pp. 507–523.
[37] L. Hommel, J.M. Geiger, M. Harms, J.H. Saurat, Sebum excretion rate in subjects treated with oral all-*trans*-retinoic acid, Dermatology 193 (1996) 127–130.