

Retinoid Metabolism in the Skin

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I. Introduction

Vitamin A (retinol) and its naturally occurring and synthetic derivatives, collectively referred to as retinoids, exert a wide variety of profound effects in embryogenesis, reproduction, vision, and regulation of inflammation, growth, and differentiation of normal and neoplastic cells in vertebrates (Sporn *et al.*, 1994; Blomhoff, 1994; Becherel *et al.*, 1994).

Vitamin A was first reported to be an essential nutrient ("fat soluble A") in the beginning of this century (Drummond, 1920). The importance of retinoids in dermatology dates back to Wolbach and Howe in 1925, who identified epidermal changes as abnormal keratinization in vitamin A-deficient animals (Wolbach and Howe, 1925). These observations were followed by numerous studies focused on the metabolism and pharmacological action of retinoids in the skin leading to the establish-

ment of retinoic acid treatment for various skin diseases (Stuettgen, 1962; Baer, 1962; Frost and Weinstein, 1969; Fredriksson, 1971; Schumacher and Stüttgen, 1971; Günther, 1973; Runne *et al.*, 1973). Up to now, far more than 5000 retinoic acid analogs have been synthesized, out of which the next three generations have been established for systemic and topical treatment of various skin disorders: first, the nonaromatic retinoids β -carotene (provitamin A), *all-trans*-retinoic acid (RA) (tretinoin), and 13-*cis*-RA (isotretinoin); second, the monoaromatic retinoid derivatives trimethyl-methoxyphenyl analog of RA (etretinate) and 9-(4-methoxy-2,3,6-trim-

^b Abbreviations: ADH, alcohol dehydrogenase; AhR, arylhydrocarbon receptor; ALDH, aldehyde dehydrogenase (RaldDH); AP1, activator protein 1; cDNA, complementary deoxyribonucleic acid; CRBP, cellular retinol binding protein (apo- and holo); CRABP, cellular retinoic acid binding protein (apo- and holo); CYP, cytochrome P450-isoenzymes; LRAT, lectin:retinol acyltransferase; mRNA, messenger ribonucleic acid; NAD, nicotinamide adenine dinucleotide; RA, retinoic acid; RAL, retinal; RaldDH, retinal dehydrogenase; RAR, retinoic acid receptor; RBP, retinol binding protein; RE, retinylesters; REH, retinylester hydrolase; ROL, retinol; RoldH, retinol dehydrogenase; RXR, retinoid X receptor; RXRE, retinoid X responsive element; SDR, short-chain dehydrogenase/reductase.

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ethylphenyl)-3-2,4,6,8-nonatetraenoic acid (acitretin); and third, the polyaromatic retinoid derivatives tazarotenic acid and 6-[3-(1-adamantyl)-4-methoxy-phenyl]-2-naphthoic acid (adapalene) (see fig. 1) (Orfanos *et al.*, 1987, 1997; Shalita *et al.*, 1996).

Retinoids mediate their biological effects through binding to nuclear receptors known as RA receptors (RARs) and retinoid X receptors (RXRs), which belong to the superfamily of ligand-inducible transcriptional regulators that include steroid hormone receptors, thyroid hormone receptors, and vitamin D₃ receptors (reviewed in: Giguere, 1994; Mangelsdorf *et al.*, 1994; Chambon, 1996). RARs and RXRs act via polymorphic *cis*-acting responsive elements, the RA responsive elements (RAREs), and retinoid X responsive elements (RXREs), present in the promoters of retinoid-responsive genes (Giguere, 1994; Mangelsdorf *et al.*, 1995; Gronemeyer and Laudet, 1996). The functional interactions of retinoid receptors in the skin were reviewed by Fisher and Voorhees (1996) and Chambon (1996).

Although *all-trans*- and *9-cis*-RA are only minor metabolites of retinol (ROL) and β -carotene, they display 100- to 1000-fold higher biological activity (Breitman *et al.*, 1980; Strickland and Mahdavi, 1978). Whereas *all-trans*-RA binds only to RARs, *9-cis*-RA binds both RARs and RXRs. The stereoisomer of *all-trans*-RA, *13-cis*-RA, exhibits a much lower affinity for RARs and RXRs and exerts its molecular effects mostly through its isomerization into *all-trans*-RA (Allen *et al.*, 1993).

Retinoids display key regulatory functions in epidermal growth and differentiation but the cellular, immunologic, and biochemical alterations associated with them are not understood completely (Fisher *et al.*, 1991; Fisher and Voorhees, 1996). Furthermore, the metabolic pathways of retinoids operative in skin physiology and pharmacotherapy remain to be defined.

In this review, the metabolic pathways of retinoids in skin are reviewed focusing on the following subjects:

1. The enzymes and binding proteins that mainly are involved in the activation, modulation, and cleavage of retinoids in human skin. The involvement of these enzymes/binding proteins in the pathogenesis of skin disorders, especially malignancies and disorders of keratinization, will be emphasized.
2. The xenobiotics that are capable of modulating the steady-state of tissue retinoid concentrations, and their impact on the enzyme systems that regulate the metabolic pathways of retinoids. Here, the "check points" in the metabolic pathway of retinoids whereby xenobiotics can influence these agents are of major interest with regard to clinical retinoid therapy.

II. Absorption, Transport, and Storage

Major sources of natural retinoids are animal fats, fish liver oil (retinylesters), and yellow and green vege-

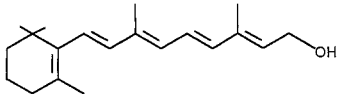
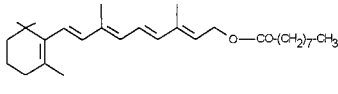
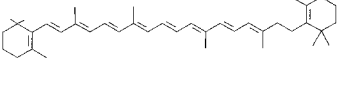
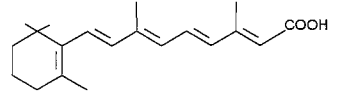
tables (carotenoids) (fig. 2). Ingested retinylesters (RE) are hydrolyzed to ROL by enteral hydrolases in the intestine. ROL and carotenoids are absorbed by intestinal mucosa cells. Of the carotenoids, β -carotene is the most potent ROL precursor, yet it is six-fold less effective than preformed ROL, which results from incomplete resorption and conversion (One ROL equivalent is equal to 1 μ g of ROL, 6 μ g of β -carotene, or 12 μ g of mixed carotenoids) (Blomhoff *et al.*, 1971).

After intestinal absorption, retinoid production from carotenoids can occur by two pathways: First, retinal (RAL) can be synthesized by oxidative cleavage of the central double bond followed by reduction to ROL by a microsomal retinal reductase (Kakkad and Ong, 1988). Here, the cellular retinol binding protein-II (CRBP-II) protects RAL from oxidation into RA. Second, apo-carotenoids are formed through excentric cleavage followed by transformation of the apo-carotenoid acids into RAs (Wang *et al.*, 1991).

In the intestinal cell, ROL also forms complexes with CRBP-II. This ROL-CRBP-II complex serves as substrate for the esterification of ROL to RE by a lecithin: retinol acyltransferase (LRAT) (MacDonald and Ong, 1988) with long-chain fatty acids, which are incorporated by chylomicrons (Blomhoff *et al.*, 1990). The fatty acids reach the general circulation where they undergo several biochemical changes via the lymph RE-chylomicron complexes. This leads to the formation of several chylomicron remnants, which in turn are cleared primarily by the liver, although extrahepatic chylomicron uptake has been shown also in bone marrow and spleen, and to a lesser degree in testes, lungs, kidneys, fat, and skeletal muscle (Blomhoff, 1994; Blomhoff *et al.*, 1991).

In the parenchymal hepatocytes, chylomicron-RE complexes are hydrolyzed and free ROL binds to retinol binding protein (RBP), its serum transport protein. Excess ROL undergoes a paracrine transfer from the hepatocytes to the perisinusoidal stellate cells, called *vitamin A storage* or *Ito cells*, for storage (Hirosawa and Yamada, 1973). Approximately 50 to 80% of the total body vitamin A in humans is stored in the stellate cells in the liver in the form of REs. Depending on their lipophilic character, exogenous and endogenous RA derivatives accumulate in the human body with highly variable elimination half-lives. This has to be considered especially for the use of synthetic RA derivatives in clinical therapy (Chien *et al.*, 1992). To maintain constant physiological ROL concentrations in the plasma of approximately 2 μ mol/L, ROL can be released from the stellate cells. The RA concentration in the plasma and other body fluids is approximately 100-fold lower (7 to 14 nmol/L) (Napoli *et al.*, 1985; De Leenheer *et al.*, 1982; Tang and Russel, 1990; Eckhoff and Nau, 1990).

ROL-RBP complexes released from the liver bind to transthyretin, a serum protein named for its ability to bind and transport simultaneously but independently

Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Retinol (Vitamin A)		Oral	Vitamin Supplement	
Retinyl Palmitate		Topical 0.5-5% Emulsions	Cosmetic Agents	
β -Carotene (Provitamin A)		Topical	Hypopigmentations, Hyperpigmentations, Radical Protection	Nutrient Color
Tretinoin		Topical 0.025-0.1% Gels or Creams	Acne vulgaris, Parakeratosis, Hyperkeratosis	Photoaging, Actinic Keratosis

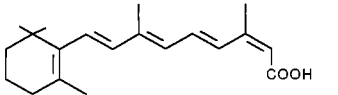
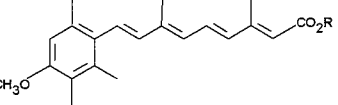
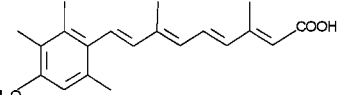
Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Isotretinoin		Topical 0.05% cream Oral 0.25-1.0 mg/kg/d	Cystic Acne, Recalcitrant Nodular Acne	Rosacea Gram-negative Folliculitis Pyoderma faciale Hidradenitis suppurativa Cancer Prevention
Etretinate		Oral 0.25-1.0 mg/kg/d	Generalized pustular Psoriasis, Exfoliative Psoriasis, Plaque Psoriasis	
Acitretine		Oral 0.25-1.0 mg/kg/d	Psoriasis (erythrodermic, pustular, and severe recalcitrant)	Palmoplantar keratoderma Pustulosis palmoplantaris, Ichthyosis, Darier's Disease, Pityriasis rubra pilaris, Lichen ruber planus

FIG. 1. Indications and mode of administration of commercially available retinoids in dermatological therapy.

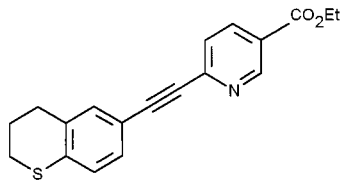
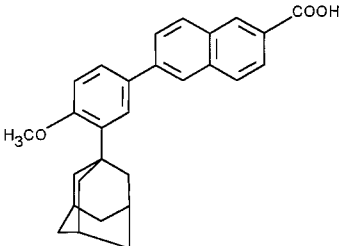
Generic Name	Chemical Structure	Mode of Administration	Principal Indication	Other Indications
Tazarotene		Topical 0.05-0.1% gels	Psoriasis vulgaris	
Adapalene		Topical 0.1% gel	Acne vulgaris	

FIG. 1. Continued.

from both the thyroid hormone and the ROL-RBP complex (Blomhoff *et al.*, 1991).

The plasma carrier of ROL, RBP, as well as the plasma carrier of RA, albumin, are present in the intercellular spaces of the epidermis (Vahlquist *et al.*, 1997; Rabilloud *et al.*, 1988). In human skin, besides ROL, β -carotene, RE, 3,4-didehydro-retinoids, RAL, *all-trans*-RA, and some of their metabolites have been identified in vitro and in vivo (Vahlquist, 1982; Vahlquist *et al.*, 1982). Cultured epidermal keratinocytes maintained in medium containing a physiological concentration of ROL exhibit a retinoid composition that is similar to intact epidermis (Randolph and Simon, 1993).

As of today, the mechanisms of ROL uptake by target cells are not understood completely. Several possibilities have been proposed: RBP receptor-mediated uptake, nonspecific spontaneous transfer of ROL and RA, and fluid phase endocytosis (Heller, 1975; Rask and Peterson, 1976; Bavic *et al.*, 1991; Dew and Ong, 1995). Orally administered *all-trans*-RA, 13-*cis*-RA, and etretinate undergo first-pass absorption directly into the portal blood and circulate in the plasma, mainly bound to albumin. The uptake of these retinoids by target cells is regulated by unknown factors. Similarly, neither the mechanism of transcutaneous absorption of topical retinoids nor their transfer into target cells is well understood.

Topically applied *all-trans*-RA is isomerized partially to 9-*cis*-RA, 13-*cis*-RA, and other metabolites within the epidermis (Lehmann and Malany, 1989). Approximately 80% of the *all-trans*-RA applied remains on the skin surface, whereas its penetration through the stratum corneum and the hair follicle is vehicle-dependent (Leh-

mann *et al.*, 1988). After the initial diffusion into the stratum corneum that occurs within a few minutes, further diffusion into epidermis and dermis proceeds more slowly (Schaefer, 1993; Tavakkol *et al.*, 1994). Our findings have shown that topically applied *all-trans*-RA and 13-*cis*-RA poorly penetrates into or through the skin: gel-based formulations tend to trap the drug and the RA remains on the surface, whereas cream formulations enhance penetration to a small extent (less than 5% of the applied amount within 30 min). On the other hand, 13-*cis*-RA penetrates rapidly into normal human keratinocytes in vitro (unpublished data).

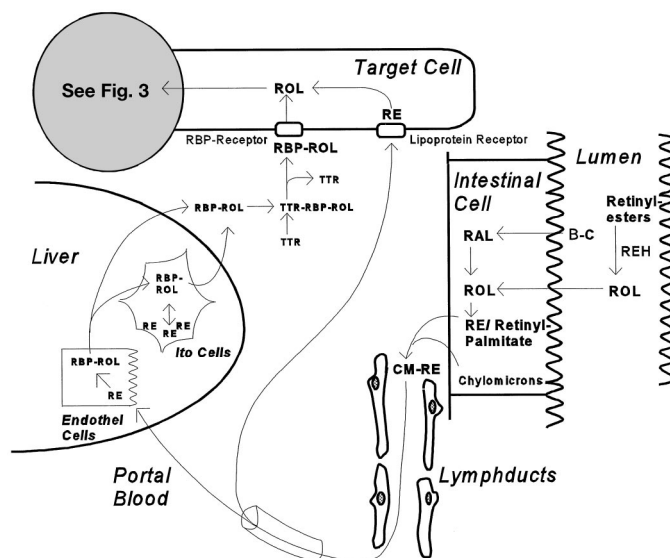


FIG. 2. Retinoid absorption, transport, and storage. B-C, β -carotene; CM-RE, chylomicron-RE complexes; RBP-ROL, RBP-ROL complexes; TTR, transthyretine.

The storage of ROL in human skin occurs through esterification of ROL into RE (Kang *et al.*, 1995). Skin cells contain transferases, LRAT and acyl CoA:acyl-transferase (ARAT). These two enzymes catalyze RE synthesis (Torma and Vahlquist, 1987; Kurlandsky *et al.*, 1996) (table 1). The hydrolysis of RE to ROL is regulated by a specific RE hydrolase. In cultured keratinocytes, LRAT activity is inducible by retinoids after 12 h incubation (Kurlandsky *et al.*, 1996). Simultaneously, biosynthesis of *all-trans*-RA is reduced, whereas inhibition of LRAT by phenylmethylsulfonyl fluoride restores *all-trans*-RA synthesis. The regulation of LRAT activity provides a mechanism of autoregulation of RA synthesis through feedback regulation of substrate availability. The esterifying activity in human skin in vivo is four-fold greater, on a per cell basis, in keratinocytes in the basal layer of the epidermis than in keratinocytes in the upper layers (Kurlandsky *et al.*, 1996), suggesting that retinoid levels are higher in the lower epidermis, which is closer to the perfusate from capillaries in the dermis. Furthermore, this implies that more REs are stored in the lower than in the upper epidermal keratinocyte layers. During migration from the lower to the upper cell layers, these stored REs may provide keratinocytes with a source of ROL and thus, a source of RA, which maturing keratinocytes are able to synthesize from ROL (Siegenthaler *et al.*, 1990a). Human keratinocytes incubated with *all-trans*-RA exhibit time- and concentration-dependent increases in RE mass, increases in the rate of RE synthesis, and decreases in RE utilization (Randolph and Simon, 1996). This clearly demonstrates that keratinocytes respond to exogenous RA by initiating feedback inhibition of endogenous production of active retinoids, sequestering extracellular substrate ROL as RE, and decreasing RE utilization. How these reactions are mediated, and to what extent nuclear retinoid receptors are involved, is not understood completely, but it is obvious that the steady-state system of intracellular retinoids is regulated by a complex feedback control system involving retinoids, several enzymes, and retinoid binding proteins.

III. Retinoid Biosynthesis

Whereas extensive and elegant work has been performed on the family of retinoid receptors, a relatively large gap exists in the knowledge of how ROL is metabolized to form active ligands.

Because ROL produces changes in skin, in vivo, similar to those produced by RA but without measurable levels of RA or irritation, ROL generally is considered a prohormone of RA, implying that ROL-induced responses in human keratinocytes are mediated by its tightly regulated conversion to RA (Kang *et al.*, 1995). These responses include increased epidermal thickening because of increased keratinocyte proliferation, expansion of intercellular spaces, compaction of the epidermal barrier, and induction of CRBP, CRABP-II, and RA 4-hydroxylase activity. From these findings it is possible that ROL may be a more efficient and natural way to deliver RA to the correct subcellular location within skin cells than direct treatment with RA (Fisher *et al.*, 1991). In support of this, it has been shown that ROL and also REs must be converted to RA to exhibit biological activity in human keratinocytes, in vitro (Kurlandsky *et al.*, 1994; Chen *et al.*, 1995a). Because of a tight enzymatic regulation of the conversion of ROL and RAL to RA, *all-trans*-RA is minimally detectable in untreated and ROL-treated human skin (Kang *et al.*, 1995). Very low levels of RA apparently are required to function as ligands to bind and activate nuclear RARs and RXRs (for review see Giguere, 1994), and the RA that is formed from ROL is hydroxylated rapidly by RA 4-hydroxylase to the metabolites 4-OH-RA and 4-oxo-RA, which exhibit a much lower retinoid receptor binding affinity (see Section V.A.).

Numerous enzymes involved in retinoid metabolism have been identified. These enzymes are members of four distinct families: Alcohol/ROL dehydrogenase (ADH/RolDH), short-chain dehydrogenase/reductase (SDR), aldehyde/RAL dehydrogenase (ALDH/RalDH), and several cytochrome P450-isoenzymes.

A. Alcohol/Retinol Dehydrogenases and Short-Chain Dehydrogenases/Reductases

The conversion of ROL to RA consists of a two-step process: First, members of the alcohol dehydrogenase (ADH I, II, and IV) (Boleda *et al.*, 1993; Yang *et al.*, 1994; Kedishvili *et al.*, 1995) and short-chain dehydrogenase/reductase enzyme families (SDR) catalyze the reversible interconversion of ROL and RAL, the rate-limiting step (Kim *et al.*, 1992; Blaner and Olson, 1994; Chen *et al.*, 1995c) (fig. 3, table 1). These ADH-isoforms metabolize *all-trans*-, *9-cis*-, and *13-cis*-retinoid isomers with reduced nicotinamide adenine dinucleotide (NAD) as co-

TABLE 1
The reversible retinol/retinal interconversion

Enzyme	Locus	Reaction	Binding protein/function
Lectin:retinol-acyltransferase (LRAT)	Microsomes	Retinol → retinyl esters	Holo-CRBP-retinol/substrate, Apo-CRBP/inhibitor
Retinyl ester hydrolase (REH)	Microsomes	Retinyl esters → retinol	Apo-CRBP/activator
Alcohol dehydrogenase classes I, II, IV, and VII (RolDH)	Microsomes	Retinol → retinal	Holo-CRBP-retinol/substrate, Apo-CRBP/inhibitor
Short-Chain dehydrogenase/reductase, microsomal retinol dehydrogenase types I, II, and III	Cytosol	Retinol → retinal	Holo-CRBP-retinal/substrate

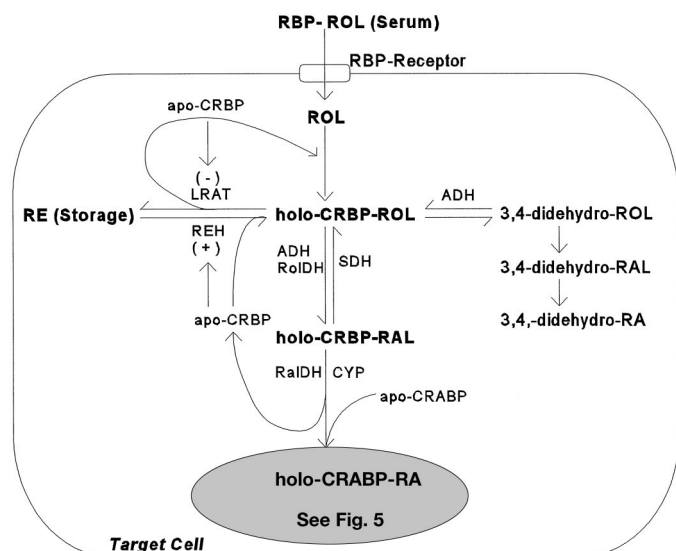


FIG. 3. Storage and metabolism of retinol in the target cell. SDH, short-chain dehydrogenase.

enzyme, whereas the SDRs use *all-trans*-ROL and *all-trans*-RAL, either free or bound to CRBP-I with reduced NAD phosphate as phosphorylated coenzyme, but are unable to oxidize 9-*cis*-ROL or 13-*cis*-ROL (Boerman and Napoli, 1995). Similar ADH enzymes metabolizing ROL into RAL were identified in differentiating keratinocytes (Siegenthaler *et al.*, 1990a) and other cell types (Posch *et al.*, 1992; Tsujita *et al.*, 1994; Chen *et al.*, 1994) in vitro, as well as in human psoriatic epidermis and at very low levels in normal human skin (Siegenthaler *et al.*, 1990b).

Recent studies have revealed that mammalian ADH is part of a complex enzyme family composed of seven evolutionarily conserved classes, each with unique properties and sites of gene expression (Jörnvall *et al.*, 1995; Duester *et al.*, 1995). In mouse skin, the enzyme catalyzing ROL oxidation has been identified as an isoenzyme of ADH class IV (Connor *et al.*, 1987; Zgombic-Knight *et al.*, 1995). To what extent the other ADH classes are active in retinoid metabolism in murine and human skin is unknown.

Three forms of rat liver microsomal ROL dehydrogenases (ROLDH, types I, II, and III) revealed sequence homology with members of the SDR family (fig. 3, table

1). The ADH and SDR enzyme families are related evolutionarily, sharing similar coenzyme binding domains, but differ in that ADH has a greater subunit molecular weight and is zinc-dependent, whereas SDR has a shorter subunit and no metal requirement (Persson *et al.*, 1995). Whether these SDRs are involved in retinoid metabolism in human skin has not yet been determined.

B. Aldehyde/Retinal Dehydrogenases and Cytochrome P450

In the second step, members of the aldehyde/RAL dehydrogenases (ALDH/RalDH) and cytochrome P450-isoenzyme families (CYP) catalyze the irreversible oxidation of RAL into RA (Duester, 1996) (fig. 3, table 2). This explains why the administration of RA to vitamin A-deficient animals results in no increase in ROL and RAL production needed for retinoid storage nor does it induce the production of the visual pigment 11-*cis*-retinal (Dowling and Wald, 1960, 1982). In mouse epidermis topical RAL is transformed into *all-trans*-RA and exerts biological activity in vivo as measured by messenger ribonucleic acid (mRNA) levels of filaggrin and loricrin (Didierjean *et al.*, 1996). Out of three characterized classes, class I ALDH showed the highest activity for the oxidation of *all-trans*-RAL and 9-*cis*-RAL to the corresponding RA isomers (Lee *et al.*, 1991; Roberts *et al.*, 1992; Labrecque *et al.*, 1995). Whether this is also true for human skin is unknown.

Whereas some members of the CYP superfamily are involved in RA synthesis, they seem to be much more important for the catabolism of active retinoid ligands, as discussed below (fig. 3, table 2). Several CYP-isoenzymes derived from rabbit liver catalyze the oxidation of RAL to RA (Roberts *et al.*, 1993; Tomita *et al.*, 1993; Raner *et al.*, 1995). Here, the most important CYP-isoenzymes in human skin apparently are CYP1A1 and CYP1A2, which are both able to oxidize *all-trans*- and 9-*cis*-RAL into the corresponding RA isomers (Roberts *et al.*, 1992; Raner *et al.*, 1995). Furthermore, the basal expression of CYP1A2 and 1A1 can be inhibited by RA in human epidermis (Li *et al.*, 1995), which suggests feedback inhibition by one of the products of these enzymes.

TABLE 2
The irreversible oxidation of retinal to retinoic acid

Enzyme	Locus	Reaction	Binding protein/function
Aldehyde dehydrogenases class I (RalDH)	Cytosol	Retinal → retinoic acid	Holo-CRBP-retinal/substrate
Cytochrome P450-isoenzymes	Microsomes	Retinal → retinoic acid	Holo-CRBP-retinal/substrate
CYP1A1		Retinal → 4-OH-retinal	
CYP1A1		9- <i>cis</i> -Retinal → 4-OH-9- <i>cis</i> -retinal	
CYP3A6		→ 4-oxo-9- <i>cis</i> -retinal	
CYP1A2		9- <i>cis</i> -Retinal → 9- <i>cis</i> -retinoic acid	
CYP2B4, CYP2C3		9- <i>cis</i> -RAL → 4-OH-retinal	

The synthesis of RA does not require ROL as a substrate (Napoli and Race, 1988). Because the cleavage of β -carotene involves RAL as an intermediate product, it can function as an alternative precursor for RA in epidermal cells and several other tissues (fig. 4) (Vahlquist, 1982; Vahlquist *et al.*, 1982; Lakshman *et al.*, 1989; Krinsky *et al.*, 1993).

Although 9-*cis*-RA is known as a ligand for RXRs and RARs, the pathway of its synthesis has not been determined completely. Evidence exists that it arises from nonenzymatic isomerization of *all-trans*-RA (El Akawi and Napoli, 1994). Other naturally occurring 9-*cis*-retinoid derivatives such as 9-*cis*-ROL or 9-*cis*- β -carotene, which have been identified in several tissues, also could function as precursors of 9-*cis*-RA (fig. 5) (Stahl *et al.*, 1993). In rat liver, high 9-*cis*-ROL dehydrogenase activity has been observed (Napoli, 1996), and the major RAL dehydrogenase equally efficiently converts *all-trans*-RAL and 9-*cis*-RAL into their respective acids, whereas it discriminates against 13-*cis*-RAL (Giguère, 1994). This 9-*cis*-RAL dehydrogenase could convert 9-*cis*-RAL, which is produced from dietary 9-*cis*-ROL or from 9-*cis*- β -carotene, into 9-*cis*-RA. Because the mechanism of *all-trans*- to 11-*cis*-isomerization is comparable with *all-trans*- to 9-*cis*-isomerization, 9-*cis*-RA also could originate from the generation of 9-*cis*-ROL from RE, as observed with 11-*cis*-ROL (Cañada *et al.*, 1990). In human plasma, 9-*cis*-RA is converted rapidly but reversibly into 9,13-*cis*-RA (Horst *et al.*, 1995). Recently, we showed that this RA derivative is present in human epidermal keratinocytes and fibroblasts, *in vitro* (unpublished data). However, the function of 9,13-*cis*-RA has yet to be determined. This interconversion may represent a mechanism for 9-*cis*-RA clearance, or similarly to the 13-*cis*-/*all-trans*-RA interconversion, for a circulating less toxic derivative of 9-*cis*-RA as a depot for later use (Napoli, 1996).

Little is known about the isomerization of retinoids, although this reaction apparently is of great importance for the maintenance of appropriate intracellular levels of active RA. The *cis*-isomers of *all-trans*-ROL, *all-trans*-RAL, and *all-trans*-RA may be produced by nonenzy-

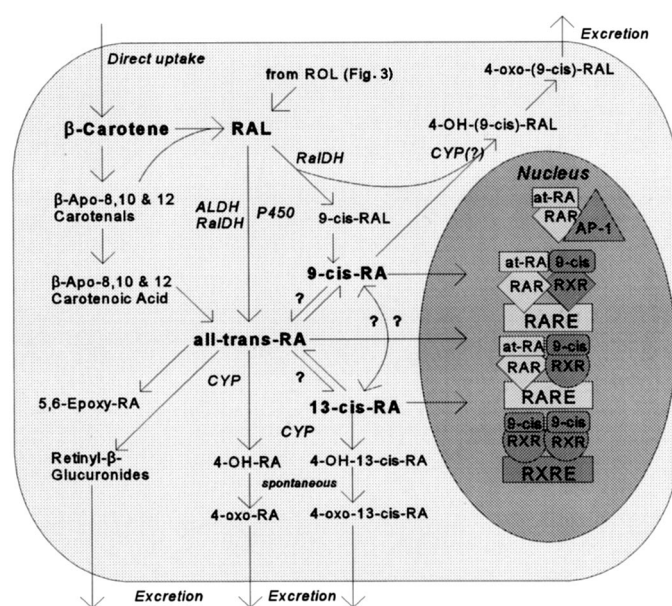


FIG. 5. Intracellular pathways of retinoid metabolism. at-RA, *all-trans*-RA.

matic isomerizations (El Akawi and Napoli, 1994; Kojima *et al.*, 1994; Urbach and Rando, 1994), by cytochrome P450-isoenzymes modifying the β -ionone ring, or by other yet unknown enzymatic conversions. The *all-trans*- to 9-*cis*-isomerization, generating the major ligand for the RXRs, occurs not only among RA isomers but also from *all-trans*-RAL to 9-*cis*-RAL, driven by a specific ADH (Labrecque *et al.*, 1995). Remarkably, 9-*cis*-RA levels in human skin are much lower than *all-trans*-RA, and 9-*cis*-RA applied topically to human skin is isomerized rapidly to *all-trans*-RA (Duell *et al.*, 1996a), suggesting the existence of an isomerase that preferentially produces *all-trans*-RA. Alternatively, 9-*cis*-RA is formed from 9-*cis*- β -carotene (Nagao and Olson, 1994; Wang *et al.*, 1994). The physiological significance of this reaction is unknown.

We observed that 13-*cis*-RA spontaneously isomerizes to *all-trans*-RA very rapidly in cell-free medium (an equal ratio was reached in less than 24 h), and a ratio of 1:2.1 after 54 h was measured. In human keratinocytes *in vitro* this isomerization is slowed (the equal ratio was reached within 36 h), and the ratio after 54 h is still less than 1:1.7. Using *all-trans*-RA as the substrate for these isomerization studies, only small amounts of 13-*cis*-RA are converted from *all-trans*-RA, indicating that *all-trans*-RA is the most stable isomer. 9-*cis*-RA is converted rapidly into 13-*cis*- and *all-trans*-RA in human keratinocytes *in vitro*. These observations suggest the existence of an enzyme regulating the interconversion of these three isoforms of RA in human keratinocytes (Jugert *et al.*, unpublished results). Investigation is underway to test this hypothesis.

In addition to several RA derivatives, human epidermis also produces 14-OH-4,14-*retro*-ROL (Duell *et al.*,

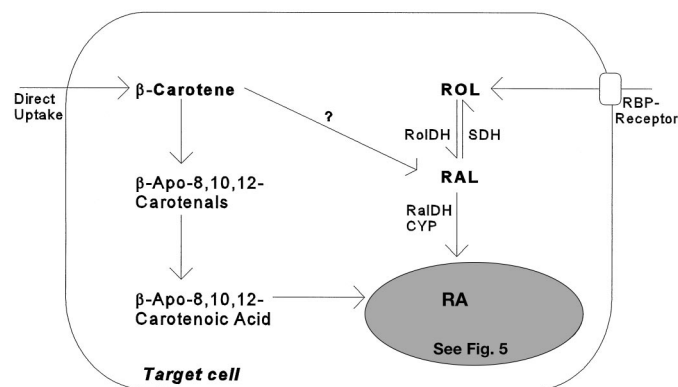


FIG. 4. Metabolism of β -carotene in the target cell.

1996b), which first was shown to be biologically active in B lymphocytes, where it substitutes for ROL in maintaining cell growth in culture (Buck *et al.*, 1991). The role of this retinoid in human skin is not clear. Similarly, the functional significance of other vitamin A derivatives, 3,4-*didehydro*-ROL (vitamin A₂) (Vahlquist, 1980), 9,13-*cis*-RA (Horst *et al.*, 1995) and 3,4-*didehydro*-RA, which are synthesized in human keratinocytes, is unknown (Randolph and Simon, 1993). The finding that topical treatment with RA decreases the concentration of 3,4-*didehydro*-retinoids in skin suggests that these metabolites may function as a retinoid storage form (Randolph, 1996). Thaller and Eichele (1990) speculated that 3,4-*didehydro*-RA may function as an endogenous morphogen, which is as important as *all-trans*-RA.

Besides this complex and not yet completely characterized enzyme system, several retinoid binding proteins interacting with both the substrates and the enzymes are also very important regulators of intracellular retinoid metabolism.

IV. Retinoid Binding Proteins

Within the cytoplasm, ROL and RA are bound to specific cellular binding proteins, CRBP-I and -II and CRABP-I and -II, respectively. These proteins are involved in the regulation of the intracellular concentration of ROL, RAL, and RA by acting as both storage or shuttle proteins in retinoid metabolism, and maintain constant cell-specific levels of free ROL and RA (fig. 3, tables 1–3). The concentrations of both CRBP-I/II and CRABP-I/II exceed those of their ligands (Harrisson *et al.*, 1987; Donovan *et al.*, 1995) and exhibit affinities for their ligands which are much higher than many enzymes for their substrates (Li *et al.*, 1991; Norris *et al.*, 1994).

A. Cellular Retinoid Binding Proteins-I and -II

CRBPs facilitate the uptake of ROL and present it to LRAT for storage as REs (Ong, 1994) (table 1). Further, they prevent ROL from spontaneous nonenzymatic isomerization and oxidation, which occur rapidly in the absence of CRBP (Napoli *et al.*, 1995). ROL bound to CRBP-I (holo-CRBP) is a substrate for conversion to RA (Posch *et al.*, 1992; Ottonello *et al.*, 1993; Boerman *et al.*, 1995), and unbound CRBP (apo-CRBP) inhibits LRAT

(Ong, 1994) (table 1). Thus, the ratio of apo- to holo-CRBP participates in regulation of the balance between oxidation and esterification of ROL (Napoli, 1993). The activity of microsomal ROL dehydrogenase (SDRs) with *all-trans*-ROL (as ROL dehydrogenase) or with *all-trans*-RAL (as RAL reductase) is stimulated in both cases by CRBP-I, facilitating the conversion of ROL to RE as shown in human liver (Yost *et al.*, 1988).

The expression of CRBP is up-regulated by both RA and ROL in several tissues, including human skin (Eskild *et al.*, 1988; Rush *et al.*, 1991; Ong *et al.*, 1994), and it has been suggested that this is caused by the conversion of ROL to *all-trans*-RA (Kurlandsky *et al.*, 1994), indicating that this induction of CRBP gene transcription by *all-trans*-RA is a negative feedback regulatory mechanism of RA synthesis (Smith *et al.*, 1991; Mangelsdorf *et al.*, 1991; Wang *et al.*, 1993; Ong *et al.*, 1994), which decreases the levels of free ROL, and thus inhibits the conversion of ROL to RA.

B. Cellular Retinoic Acid Binding Proteins-I and -II

The intracellular levels of CRABP-I protein are similar in dermis and epidermis, whereas CRABP-II levels are much higher in the epidermis (Siegenthaler *et al.*, 1984; 1992b). Furthermore, CRABP-II is up-regulated by treatment with ROL (Kang *et al.*, 1995), *all-trans*-RA and its analogs (Astrom *et al.*, 1991; Elder *et al.*, 1992), especially in differentiating keratinocytes. These findings have led to the use of the CRABP-II response to retinoid administration in fibroblasts in vitro as a reproducible measure of retinoid bioactivity that may predict human skin responses (Elder *et al.*, 1996). The sources of CRABP-II in human skin are keratinocytes and fibroblasts, whereas the source of CRABP-I in human skin is primarily melanocytes (Sanquer and Gilchrest, 1994). Basal CRABP-I expression is much lower than that of CRABP-II.

CRABP-I and -II display variable RA binding affinities, regulated by a RA-responsive element in their promoters (Darmon and Blumenberg, 1993). By this mechanism, CRABP-I has been implicated in enhancing the metabolism of RA to inactive metabolites (Fiorella and Napoli, 1991) through a transfer of RA from CRABP-I to CYP-isoenzymes via a bimolecular complex with juxtaposed ligand portals (Thompson *et al.*, 1995), whereas CRABP-II may facilitate transport of RA to the nucleus

TABLE 3
The catabolism of retinoic acid to polar metabolites

Enzyme	Locus	Reaction	Binding protein/function
Cytochrome P450-isoenzymes	Microsomal		
P450RAI		Retinoic acid → 4-OH-retinoic acid	CRABP-retinoic acid/substrate
CYP1A1		Retinoic acid → 4-OH-retinoic acid	CRABP-retinoic acid/substrate
CYP1A2			
CYP2B4			
CYP2C3			
CYP2E1, 2			
CYP2G2			
CYP3A			

(Donovan *et al.*, 1995), suggesting that CRABP-II is involved in the regulation of nuclear receptors by RA. F9-cell mutants that overexpress CRABP-I show a much faster RA metabolic activity than wild-type F9 cells, and much higher RA concentrations are required to induce differentiation (Boylan and Gudas, 1991).

Here it should be mentioned that 13-*cis*-RA is not bound by CRABP-I/II or other binding proteins in the cytosol. Thus it penetrates the cell nucleus after topical or systemic administration more rapidly than *all-trans*-RA or 9-*cis*-RA, as demonstrated in a mouse embryo model system, whereas the access of *all-trans*-RA to the nucleus seems to be limited by its binding to CRABP-I/II (Nau and Elmazar, 1997). These findings may explain the profound teratogenic effects caused by 13-*cis*-RA after topical or systemic treatment (Orfanos *et al.*, 1997). The kinetics of the nuclear penetration of RA derivatives in human skin cells is currently under study in our laboratory.

A significant decrease of CRABP-I mRNA expression and an increased CRABP-II mRNA expression have been reported in psoriatic skin (Siegenthaler *et al.*, 1990a, 1992a; Elder *et al.*, 1992; Torma *et al.*, 1994). Moreover, the expression of CRBP-I mRNA also was increased (Busch *et al.*, 1992). Whether this altered expression of CRBP-I and CRABPs is an inherent characteristic of psoriasis or simply reflects the fact that psoriatic epidermis contains a higher proportion of undifferentiated keratinocytes is unknown.

CRABP-I expression was down-regulated in basal and squamous cell carcinomas, whereas CRBP-I was expressed (Busch *et al.*, 1992). Whether these findings are relevant to the development of these skin tumors is not known.

CRABP-I/II double knockout mice had no apparent phenotype (Lampron *et al.*, 1995), suggesting that these binding proteins may not be essential for normal retinoid metabolism or signaling. Instead, it is possible that these proteins sequester RA during vitamin A deficiency to support the maintenance of retinoid signaling. However, retinoid binding proteins (CRBPs and CRABPs) are involved in the regulation of intracellular retinoid concentrations (tables 1–3) and display atypical patterns in psoriasis and other hyperproliferative skin diseases. To what extent the phenotype of these skin disorders is caused by an inappropriate metabolism of retinoids, and whether the atypical patterns of retinoid binding proteins found are primary or secondary, remains to be elucidated. Further investigation is underway to characterize the function of CRBPs and CRABPs in healthy, psoriatic, and neoplastic skin.

V. Retinoid Catabolism

A. Retinoic Acid Metabolites

The cleavage of active retinoid ligands to inactive metabolites is of great importance for the regulation of

nuclear retinoid receptors (fig. 5). Recently, the growth inhibitory effects of RA have been shown to correlate with the activity of RA metabolism (Takatsuka *et al.*, 1996). The enzyme mainly responsible for this reaction is the CYP-dependent 4-hydroxylase that converts the β -ionone ring of RA to 4-*hydroxy*-RA metabolites (4-OH-RA) which are excreted much faster from the cells than RA (Roberts *et al.*, 1979; Williams and Napoli, 1985; Westin *et al.*, 1993). The CYP dependence of this reaction has been demonstrated in microsomes of rat skin (Van den Bossche *et al.*, 1988), rabbit (Roberts *et al.*, 1992) and human liver (Leo *et al.*, 1989), human keratinocytes (Roos *et al.*, 1996), and human skin (Duell *et al.*, 1994).

Interestingly, *all-trans*-, 9-*cis*-, and 13-*cis*-RA induce a 4-hydroxylase, which seems to metabolize only *all-trans*-RA (Duell *et al.*, 1994, 1996) in human skin. Conversely, 9-*cis*- and 13-*cis*-RA inhibited the 4-hydroxylation of *all-trans*-RA in human liver (Nadin and Murray, 1996). However, topical application of pharmacological doses of *all-trans*-RA to human skin induces a 4.5-fold increase in its metabolism to 4-OH-RA and other polar metabolites (Duell *et al.*, 1992). Our findings showing that both 9-*cis*- and 13-*cis*-RA are isomerized to *all-trans*-RA in human keratinocytes, *in vitro*, may explain why topical application of these substances to human skin induces *all-trans*-RA 4-hydroxylase, leading to an increase in 4-OH-metabolites in epidermis regardless of the isomer applied (Jugert *et al.*, *in press*). In contrast, human fibroblasts show no significant RA isomerization activity regardless of the isomer (13-*cis*), 9-*cis*, and *all-trans*-RA, respectively) applied. The major catalytic metabolite identified in fibroblasts is 4-oxo-13-*cis*-RA, no matter which RA isomer is added to the medium (unpublished results). Other studies have shown that microsomal preparations from mouse liver but not mouse skin can 4-hydroxylate 13-*cis*-RA in an *in vitro* assay system (Oldfield, 1990). In contrast, in cooperation with R. Wyss (Roche Laboratories, Basel, Switzerland), we found that RA 4-hydroxylase in liver and skin of National Marine Research Institute mice is cytochrome CYP 2E1 (CYP2E1) dependent. After induction of the CYP2e1-specific *para*-nitrophenol hydroxylase by the addition of ethanol to drinking water or the application of ethanol to mouse skin, we observed a parallel increase in *p*-nitrophenol hydroxylase activity and the amount of 4-hydroxylated RA metabolites, indicating that CYP2e1 is a major RA 4-hydroxylating enzyme in murine liver and skin (Jugert *et al.*, 1995, 1996a). An alternative pathway of 13-*cis*-RA inactivation after topical application has been proposed through absorption into the bloodstream and transport to the liver for conversion to 4-OH-13-*cis*-RA by hepatic CYP2c8 (Leo *et al.*, 1989).

4-oxo-*all-trans*-RA is the best characterized RA metabolite. It is approximately half as active as *all-trans*-RA in promoting cell differentiation in F9 embryonal carcinoma cell lines (Williams *et al.*, 1992) and in producing dysmorphogenic effects in rat embryonal tis-

sue (Kraft *et al.*, 1992). Likewise, 4-oxo-RA binds RAR β with an affinity similar to that of *all-trans*-RA (Pijnappel *et al.*, 1993), but binds poorly to RAR γ (Reddy *et al.*, 1992). 4-oxo-RA also binds to CRABP with an affinity slightly lower than that of *all-trans*-RA in vitro (Fiorella *et al.*, 1993)

Our experiments have shown that the isomers 13-*cis*-RA and *all-trans*-RA are predominantly inside the keratinocytes, whereas their 4-OH- and 4-oxo-metabolites are excreted rapidly from the cells, indicating that these metabolites do not bind appreciably to receptors (unpublished observations).

The formation of 4-oxo-RA from 4-OH-RA was shown to require only NAD, whereas reduced NAD phosphate was ineffective, which is inconsistent with the involvement of CYP-isoenzymes (Allenby 1993). Wyss (personal communication) observed that there are no differences in the oxidizing activity of 4-OH-RA in cultured human keratinocytes and fibroblasts compared with the tissue culture medium, which suggests that 4-OH-RA is oxidized spontaneously to 4-oxo-RA depending on the concentration of *all-trans*-RA added to the culture medium and the induction/inhibition of the RA 4-hydroxylase. Alternatively, the formation of 4-oxo-RA can result from oxidation at the 2 or 3 position of the β -ionone-ring of 4-OH-RAL, catalyzed by a CYP1A2-mediated 4-oxidation of *all-trans*-RAL and 9-*cis*-RAL, and not from RA, as is assumed generally (Van Wauwe *et al.*, 1994; Raner *et al.*, 1996). This could be an alternative pathway for generation of 4-oxo-RA but would not completely explain how RA is cleaved.

Another group of RA-metabolites, the retinoyl- β -glucuronides (*all-trans*-RAG, 13-*cis*-RAG, and 9-*cis*-RAG), also may have retinoid receptor binding activity as demonstrated in vivo and in vitro (Mehta *et al.*, 1991; Olson *et al.*, 1992; Sass *et al.*, 1994). Only traces of RA-glucuronides are found in human tissues compared with rodents (Sass *et al.*, 1994). This species difference in glucuronidation could explain the high clearance of 13-*cis*-RA in rodents compared with humans (Nau *et al.*, 1989; Nau, 1990).

The natural metabolite of RA, 5,6-epoxy-RA, is found in intestinal mucosa, liver, and kidney of rat (Napoli and McCormick, 1981). Although this metabolite inhibits the promotion of skin tumors equipotent with RA (Verma *et al.*, 1980), it is converted to polar metabolites more rapidly than RA (Napoli *et al.*, 1982). The specific enzyme generating 5,6-epoxy-RA is unknown.

Using a Cat-reporter assay (Astrom *et al.*, 1990) and ED₅₀ values for RXR α and RAR γ , a potency grading of retinoid binding activity and receptor inducibility has been evaluated with *all-trans*-RA > *dd*-RA > 4-oxo-RA > 4-OH-RA > 5,6-epoxy-RA (Duell *et al.*, 1992). These metabolites produce epidermal thickening in hairless mouse skin in a rank order similar to that achieved with the Cat assay (Reynolds *et al.*, 1993).

B. Cytochrome P450-Isoenzymes

Several isoenzymes of the cytochrome CYP superfamily are involved specifically in the catabolism of retinoids in rodent liver (Frolik *et al.*, 1979; Roberts *et al.*, 1979; Leo *et al.*, 1984; Martini and Murray, 1993; Raner *et al.*, 1996), trachea (Frolik *et al.*, 1979), intestine (Roberts *et al.*, 1991), and skin (Leo *et al.*, 1984; Van den Bossche *et al.*, 1988) (table 3).

White *et al.* (1996) reported the identification of RA-inducible *all-trans*-RA 4-hydroxylase (CYPRAI), encoding a new member of the cytochrome P450 supergene family in zebrafish. They found that this gene was related closely to a human complementary deoxyribonucleic acid (cDNA) isolated from a human fetal brain library, suggesting that this novel CYP subfamily is highly conserved in fish and humans, and identified the cDNA representing this RA-inducible enzyme in various human tissues as a novel family of the CYP superfamily, named CYP26 (White *et al.*, 1997). To what extent this CYPRAI is involved in the regulation of retinoid signaling in human skin cells is unknown.

Various cytochrome CYP-isoenzymes are involved in the catabolism of RA in rodent and human tissues (table 3): In rabbit liver microsomes, cytochrome CYP-1a2 (CYP1A2) and 2B4 were most effective in metabolizing RA to 4-OH-RA, whereas CYP2c3, 2E1, 2G2, and 2E2 were less effective, and CYP1A1 and 3A6 were ineffective (Roberts *et al.*, 1991). In further studies, Roberts *et al.* (1992) reported that the CYP1a2 shows high activities in both the 4-hydroxylation of RA and the oxidation of RAL to RA, whereas CYP3A6 catalyzes only the latter, and the CYP2B4 catalyzes only the 4-hydroxylation of RA.

Also in rabbit liver microsomes, Raner *et al.* (1995) demonstrated that CYP1A1 and CYP1A2, to a lesser extent, are the most active enzymes in the conversion of *all-trans*-RAL, 9-*cis*-RAL, and 13-*cis*-RAL to their corresponding RA isomers. This indicates that CYP1A1 and CYP1A2, to a lesser extent, are more involved in activation than in catalytic oxidation of retinoids. The k_{cat}/K_m value for 4-hydroxylation of *all-trans*- and 13-*cis*-RAL by CYP1A1 is identical with that for *all-trans*-RA and 13-*cis*-RA formation, suggesting a dual role for this cytochrome in the oxidation of *all-trans*-RAL and *all-trans*-RA.

In human liver, a member of the CYP2C family was reported to catalyze the 4-hydroxylation of RA (Leo *et al.*, 1989; Duell *et al.*, 1992), whereas studies with CYP-inhibiting immunoglobulin G antibodies in rat liver revealed that the CYP3A subfamily is involved in this process but can not be considered the "principal RA 4-hydroxylase" (Martini and Murray, 1993).

Isoenzymes of CYP families 2, 3 (Van Pelt *et al.*, 1990), and 4 (Uchida *et al.*, 1997) were shown to be present in human epidermal foreskin keratinocytes. The role of

these enzyme families especially with regard to retinoid metabolism is unknown.

Cultured human keratinocytes have been shown to contain CYP1A1 (Berghard *et al.*, 1990) and the 4-hydroxylation of RA increases because of induction of CYP1A1 by 3-methylcholantrene (Van den Bossche and Willemsens, 1991; Edes *et al.*, 1991), whereas RA inhibited the catalytic activity of CYP1A1 in human skin (Li *et al.*, 1995), indicating the involvement of CYP1A1 in 4-hydroxylation of RA in this tissue.

The principal RA 4-hydroxylase for the catabolism of RA in human skin is still unknown. Whether it is the 4-hydroxylase described by White *et al.* (1996, 1997) remains to be proved. Further, it would be interesting to determine whether the basal and inducible activity of this enzyme is altered in skin diseases where RA treatment is effective (e.g., psoriasis, squamous cell carcinoma, Darier's disease, acne, solar keratosis). In the skin of patients suffering from such diseases, it also would be very interesting to evaluate which xenobiotics induce/inhibit the activity of this CYP-isoenzyme and how this affects the clinical appearance of retinoid-sensitive skin diseases.

Because a range of CYP-isoenzymes seem to possess some capacity to 4-hydroxylate retinoids, attempts to isolate the principal RA 4-hydroxylase on the basis of CYP-isoenzyme activity should be conducted carefully.

The extent to which RARs and RXRs are involved in the regulation of retinoid-metabolizing enzyme activity in human skin is unknown and currently is being studied using dominant negative mutants of several RARs and RXRs.

VI. Modulation of Retinoid Metabolism: Pharmacological Interactions

A. Retinoids and Skin Malignancies

Actinic keratoses were the first skin lesions to be treated topically with *all-trans*-RA (Stüttgen, 1962). In various clinical trials, retinoids have been shown to be active in chemoprevention and treatment or prevention of skin malignancies (Verma, 1987; Hong *et al.*, 1990; Crowe *et al.*, 1991; Hu *et al.*, 1991; Houle *et al.*, 1991; Jones *et al.*, 1992; Moon and Mehta, 1990; Bertram, 1993; Reynolds *et al.*, 1993; De Luca *et al.*, 1993; Lotan *et al.*, 1995; Craven and Griffiths, 1996; Agarwal *et al.*, 1996). These effects are assumed to relate to RAR-mediated antipromoting (Hill and Grubbs, 1992) and anti-initiating effects. The latter seems to be influenced by interference of several xenobiotics with different steps of the metabolism of retinoids in liver and skin microsomes (Verma, 1992; De Luca *et al.*, 1994).

Some well known skin procarcinogens, such as 3-methylcholantrene (Kinoshita and Gelboin, 1972; Van den Bossche and Willemsens, 1988, 1991) and the polycyclic aromatic hydrocarbon benzo[a]pyrene (Falk *et al.*, 1964; Van den Bossche and Willemsens, 1991; Davies,

1967; Bickers and Kappas, 1978; Edes *et al.*, 1991), can increase RA catabolism in human skin and induce local tissue depletion of retinoids, respectively (Edes *et al.*, 1991). This can be antagonized by high dietary intake of β -carotene (Edes *et al.*, 1991) or RA (Li *et al.*, 1995). This acceleration of retinoid cleavage primarily is caused by the xenobiotic-mediated induction of CYP1A1, which also is involved in the inactivation of RA to 4-OH-RA (Van den Bossche and Willemsens, 1991; Edes *et al.*, 1991; Kizaki *et al.*, 1996). Accordingly, retinoid-induced inhibition of basal as well as coal tar- and glucocorticoid-induced CYP1A1 expression in human skin, as reported by Li *et al.* (1995), seems to reflect a competitive feedback-inhibition of CYP1A1 activity by RA.

CYP1A1 is one major enzyme that converts the procarcinogens mentioned above into active carcinogenic metabolites in skin (Bickers and Kappas, 1978). The induction of this enzyme, leading to an acceleration of the turnover of RA to inactive metabolites and a local RA deficiency, might explain further the profound effect of these carcinogenic CYP1A1-inducers on cell proliferation and tumor formation. In support of this notion, 7,8-benzoflavone, an inhibitor of CYP1A1 activity, increases local vitamin A concentrations and reduces tumor formation in mouse skin (Gelboin *et al.*, 1970). To what extent the procarcinogenic effects of these substances are caused by their induction of CYP-mediated depletion of retinoid levels in the skin, and which CYP-isoenzyme besides CYP1A1, especially the CYP1A2 (White *et al.*, 1996, 1997), are involved is unknown. However, the capacity of RA to down-regulate basal as well as inducible CYP1A1 expression indicates that retinoids also have, besides their well known antipromoting potential, an anti-initiating potential by suppressing CYP1A-dependent procarcinogen activation and subsequent tumor formation in target tissues. This down-regulation may be mediated through a retinoid-responsive element in the promoter region of the human CYP1A1 gene (Vecchini *et al.*, 1994).

RA suppresses the expression of the aryl hydrocarbon receptor (AhR) in high calcium transformed cells (Wanner *et al.*, 1996). To what extent this RA-mediated receptor modulation is of importance for the differentiation of epidermal keratinocytes is not known but suggests that RA is able to influence AhR, the main regulatory element of the procarcinogen-activating enzyme CYP1A1.

The imidazole antimycotics, ketoconazole, clotrimazole, and miconazole are all well known inhibitors of various cytochrome P450-isoenzymes, affecting also the metabolism of retinoids. They first were shown to inhibit the metabolism of RA in F9 embryonal carcinoma cells (Williams and Napoli, 1987). When tested in vitro, liarsazole, a potent CYP inhibitor (Van Wauwe *et al.*, 1993), suppressed neoplastic transformation and up-regulated gap junctional communication in murine and human fibroblasts (Acevedo and Bertram, 1995), which appar-

ently was caused by the presence of retinoids in the serum component of the cell culture medium (Rogers *et al.*, 1990; Zhang *et al.*, 1992). Furthermore, liarazole magnified the cancer chemopreventive activity of RA and β -carotene in these experiments by inhibiting RA catabolism as demonstrated by absence of a decrease in RA levels in the culture medium in the presence of liarazole during 48 hours, whereas without liarazole 99% of RA was catabolized. In vivo treatment with liarazole and ketoconazole reduced the accelerated catabolism of retinoids and increased the mean plasma *all-trans*-RA concentration in patients with acute promyelocytic leukemia and other cancers (Rigas *et al.*, 1993).

The use of low-dose *all-trans*-RA in tandem with liarazole may enhance therapeutic retinoid levels in target tissues by inhibiting RA catabolism. Retinoid catabolism is induced after long-term *all-trans*-RA treatment (Lefebvre *et al.*, 1991; Muindi *et al.*, 1992) leading to RA-resistant disease (Warrell *et al.*, 1993).

B. Retinoid Resistance

Two possible explanations for accelerated clearance of retinoids in patients during long-term treatment with retinoids have been suggested (Kizaki *et al.*, 1996).

First, RA-mediated induction of CRABP expression, which lowers the plasma and intracellular levels of active RA by binding RA (see Section IV.B.), and second, the RA-mediated induction and/or constitutive overexpression of P-glycoprotein, which is encoded by the multidrug resistance gene (MDR1), leading to decreased intracellular levels of RA by enhancing active transport of intracellular retinoids out of the target cells (Hamana and Tsuruo, 1986; Chen *et al.*, 1986; Delva *et al.*, 1993).

The RAR β_2 seems to be of great importance for the retinoid-mediated regulation of epithelial cell growth and differentiation, tumor formation, and the aging process (Houle *et al.*, 1991, 1993; Gebert *et al.*, 1991; Chen *et al.*, 1995b; Lee *et al.*, 1995; Lotan *et al.*, 1995; Si *et al.*, 1996; Bartsch *et al.*, 1992, 1996). Because it is the most tightly RA-regulated retinoid receptor (Kato *et al.*, 1992), RAR β_2 appears to be essential for pathological tissue alterations in vitamin A deficiency. Whereas vitamin A deficiency causes no significant changes in the expression levels of RAR α and RAR γ mRNAs, the level of RAR β transcripts is decreased greatly in various tissues of vitamin A-deficient rats and is rapidly inducible by administration of RA (Kato *et al.*, 1992). These findings may indicate that a xenobiotic-driven depletion of retinoids favors the formation of dysplastic tissue formation or even malignant cell growth through the depletion of the RAR β_2 activity. Because the regulation of the RAR β_2 in epithelial tissue depends primarily on the expression of other retinoid receptors, especially RAR α (Schon and Rheinwald, 1996; Geisen *et al.*, 1997), the possible effects of RAR β_2 depletion on cell growth and differentiation is difficult to analyze separately from the

context of expression patterns of other retinoid receptors or other transcription factors (e.g., AP1).

In addition, vitamin D₃ and retinoids can inhibit synergistically the growth and progression of squamous cell carcinomas and actinic keratoses in chronically sun-exposed skin (Majewski *et al.*, 1997). One reason for this synergism may be the direct influence of vitamin D₃ on the isomerization and the metabolism of RA, which we observed in human keratinocytes (Jugert *et al.*, 1997). Here, vitamin D₃ inhibits the isomerization of 13-*cis*-RA to the more receptor active *all-trans* and 9-*cis*-isomers. Moreover, we found that the vitamin D₃ derivative secocholestra-trien-1,3,24-triol (tacalcitol), used for the treatment of severe keratinizing disorders, significantly inhibits 4-hydroxylation of *all-trans*-RA (Jugert *et al.*, 1998).

Further investigations are underway to elucidate these mechanisms in the control of retinoid levels in retinoid-responsive malignant skin diseases.

C. Retinoids and Disorders of Keratinization

The use of topical and oral retinoids for the treatment of disorders of keratinization, such as psoriasis and Darier's disease, has been established (Orfanos *et al.*, 1972, 1973, 1987; Runne *et al.*, 1973; Peck *et al.*, 1978; Happle *et al.*, 1987; Blanchet-Bardon *et al.*, 1991). Systemic retinoid therapy often is combined with topical drugs such as corticosteroids, dithranol, tar, and also ultraviolet A/ultraviolet B phototherapies, in which synergistic effects have been reported (Orfanos *et al.*, 1997).

Ethanol treatment of rats results in enhanced microsomal catabolism of *all-trans*-RA to 4-OH-RA and 4-oxo-RA (50%, $P < 0.01$) accompanied by increased microsomal CYP concentrations (34%, $p < 0.005$) (Sato and Lieber, 1982). This induction in turn significantly decreased the storage of ROL in the liver in baboons and rats (Sato and Lieber, 1981). One potential target of ethanol action may be CYP2E1, which oxidizes ethanol (Ohnishi and Lieber, 1977) and 4-hydroxylates retinoids (Roberts *et al.*, 1991).

Ethanol also inhibits ADH-catalyzed ROL oxidation in vitro (Julià *et al.*, 1986), and ethanol treatment of mouse embryos has been demonstrated to reduce endogenous RA levels (Deltour *et al.*, 1996). The inhibition of cytosolic RolDH activity and stimulation of microsomal RolDH activity could explain ethanol-mediated vitamin A depletion, apart from ADH-isoenzymes (Napoli, 1996). Although the exact mechanism of inhibition of retinoid metabolism by ethanol is unclear, these observations are consistent with the finding that patients with alcoholic liver disease have depleted hepatic vitamin A reserves (Leo and Lieber, 1982).

In addition to its influence on psoriasis by inhibition of RA synthesis, ethanol may exert its effects in fetal alcohol syndrome by the same mechanism, because the class IV ADH was found to play a crucial role leading to reduced RA levels after ethanol treatment in cultured mouse embryos (Deltour *et al.*, 1996). Ethanol occasion-

ally provokes acute exacerbations of psoriasis (Poikolainen *et al.*, 1990; Frank and Lentner, 1996). Because retinoids have been very beneficial in the treatment of psoriasis, an ethanol-induced decrease of intracellular ROL and RA could be one explanation for this acute worsening of psoriasis.

Liarazole has been demonstrated to be an active antipsoriatic drug (Dockx *et al.*, 1995; De Doncker *et al.*, 1991). By suppressing the CYP-mediated 4-hydroxylation of RA to 4-OH-RA, liarazole increases serum levels of RA from nearly undetectable levels to 2.9 ± 1 ng/ml serum, which enhances the action of RA in cellular differentiation (Van Wauwe *et al.*, 1994). Because liarazole is 2 to 15 times more potent than clotrimazole, miconazole, and metyrapone in inhibiting RA metabolism, it has been used successfully for the treatment of psoriasis (Dockx *et al.*, 1995).

To what extent imbalances in retinoid metabolism are responsible for the pathogenesis of psoriasis and other keratinizing disorders, and which steps of this metabolic pathway are affected, is unknown. The mechanisms of the effect of retinoid therapy in other keratinizing disorders [e.g., ichthyosis, Darier's disease, palmoplantar keratodermas, and pityriasis rubra pilaris (Borok and Lowe, 1990; Peck and Yoder, 1976; Happle *et al.*, 1987)] are unknown. Also, it is possible that the effectiveness of systemic and topical retinoids in acne could be influenced by the concomitant administration of liarazole.

D. Other Modulators of Retinoid Metabolism

The corticosteroid dexamethasone, the macrolide antibiotic triacetyloleandomycin, and phenobarbital are all well established inducers of the CYP3A subfamily (Waxman *et al.*, 1985; Wrighton *et al.*, 1985; Hostetler *et al.*, 1987; Jugert *et al.*, 1994) and can increase microsomal 4-hydroxylation of RA in rat liver (Martini *et al.*, 1993). Whether the CYP3A subfamily and its modulation by xenobiotics is important for retinoid metabolism in human skin remains to be clarified. However, CYP3A mRNA is strongly inducible in human hepatocytes with retinoid treatment *in vitro* (Jurima-Romet *et al.*, 1997).

Glucocorticoids (clobetasol) also induce the expression of CYP1A1 in human skin (Li *et al.*, 1995). This is mediated through glucocorticoid receptor responsive elements that have been identified in the first intron of the rat and human CYP1A1 genes (Hines *et al.*, 1988). These findings suggest the possibility that skin changes caused by long-term treatment with topical or systemic glucocorticoids could be mediated by a steroid-induced depletion of active retinoids. Therefore, we hypothesize that tandem treatment of patients with both glucocorticoids and low-dose RA may prevent some steroid side effects. This idea already has been confirmed in a mouse model (Schwarz *et al.*, 1994). Retinoids may have a steroid-sparing effect (Orfanos *et al.*, 1997). Investigation is underway to test whether this is related to corticoste-

roid-induced inhibition of CRABP-II expression (Piletta *et al.*, 1994).

Studies on ADH inhibitors have revealed further evidence that this enzyme functions in ROL oxidation for RA synthesis. The ADH inhibitor 4-methylpyrazole can inhibit the conversion of ROL to RA in mouse embryos *in vivo* (Collins *et al.*, 1992), whereas microsomal ROL dehydrogenases (SDHs) are not inhibited by 4-methylpyrazole (Chai *et al.*, 1995).

Exogenous fatty acids may be another remarkable way to alter the metabolism of active retinoids in cultured human epidermal keratinocytes. Randolph and Simon (1995) demonstrated that unsaturated 16- and 18-carbon fatty acids exert the following effects on intracellular retinoid metabolism: The total cell retinoid mass increases up to 50% because of RE accumulation corresponding to the added fatty acid, whereas the utilization of endogenous RE decreased up to 80%. Furthermore, the steady-state cellular concentrations of ROL, 3,4-didehydro-ROL, and their respective carboxylic acids decreased up to 80%, whereas the RA metabolism was not altered.

VII. Conclusions and Perspectives

A. Retinoid-Drug Combinations in Dermatologic Therapy

A broad spectrum of drugs is used in combination with retinoids for the treatment of dermatological disorders to enhance the efficacy of either agent. Especially in the treatment of psoriasis, several strategies have been developed whereby retinoids are combined with other agents such as selective ultraviolet irradiation, ultraviolet A irradiation with concomitant psoralen treatment, cyclosporin, vitamin D₃-derivatives, azole derivatives, urea, tar, salicylic acid, and dithranol (Gollnick, 1996; Orfanos *et al.*, 1997).

These regimens additively or synergistically may modulate the disease process and also provide opportunities to alter the regimens, especially during long-term treatment to decrease drug toxicities or to enhance efficacy. At least three types of combination strategies for retinoid-drug combinations in dermatological therapy can be described.

First, combinations of drugs displaying distinct effects on cell proliferation/differentiation and immunomodulation (e.g., retinoids and chemotherapy in advanced cutaneous T-cell lymphoma (Gollnick *et al.*, 1981; Thstrup-Petersen *et al.*, 1988)).

Second, a combination of retinoids with ultraviolet A or B radiation (and other drugs). For example, ultraviolet A irradiation with concomitant retinoid and psoralen treatment therapy (and psoralen and ultraviolet A combination) is currently one of the most effective regimens for recalcitrant severe psoriasis (Saurat *et al.*, 1988; Tanew *et al.*, 1991).

Third, drugs with metabolic interactions that can enhance the half-life of active compounds. An example of this regimen is the interaction between azole (Kato *et al.*, 1992; Van Wauwe *et al.*, 1993; Dockx *et al.*, 1995; Majewski *et al.*, 1997) and vitamin D derivatives (Gollnick, 1996; Jugert *et al.*, 1997) that inhibit the metabolism of retinoids in skin cells leading to increased intracellular amounts of active RA isomers. Further study and the identification of novel interactions of this type of drug interaction is of great clinical interest because they may decrease the dose of retinoids required for efficacy, thereby also reducing the risk of side effects of the retinoids.

The complexity of the metabolic pathways for retinoids and the likelihood that these are altered by diseases affecting the skin suggest that such novel strategies will be forthcoming. Multiple studies are underway to define the steps of retinoid metabolism where the use of modulating drugs might influence the results of dermatological therapy thereby leading to the most profound effects with regard to the clinical outcome.

B. Retinoid Receptor Agonists/Antagonists

Synthetic retinoid receptor-selective agonists/antagonists offer another new approach. This concept of drug development is based on the findings that retinoid receptors (RARs and RXRs) can target different genes depending on the activated retinoid receptor complexes in human skin (Fisher and Voorhees, 1996, 1996). The multiplicity of these retinoid signaling pathways affords potential for therapeutic opportunity as well as undesired side effects associated with retinoid therapy. It is possible that the indiscriminate activation of all pathways by nonspecific retinoid ligands could lead to unacceptable side effects so that any enhanced efficacy would be obtained at the cost of enhanced toxicity. The development of ligands selective for individual receptor subtypes relevant to a targeted disease could decrease these toxic effects and thereby improve the therapeutic index. Two new arotinoids are now available for topical use in skin diseases. These are tazarotenic acid (tazarotene) and 6-[3-(1-adamantyl)]-4-methoxyphenyl-2-naphthoic acid (adapalene) (fig. 2); other synthetic retinoid derivatives are being developed (Klein *et al.*, 1996; Duvic *et al.*, 1997).

The first of these synthetic receptor-selective ligands available for topical treatment of psoriasis is tazarotene (fig. 2), an acetylenic third-generation retinoid derivative (Esgleyes-Ribot *et al.*, 1994). It is a poorly absorbed, nonisomerizable arotinoid, which is metabolized rapidly to its free carboxylic acid, tazarotenic acid, binding with high affinity to RARs, with the rank order of affinity being $RAR\beta > RAR\gamma \gg RAR\alpha$ (Nagpal *et al.*, 1995). It does not bind to any of the RXRs. This retinoid derivative is said to have lower cytotoxic effects than other retinoids, but it achieves sustained therapeutic efficacy

in the treatment of plaque-type psoriasis (Chandraratna, 1996; Weinstein, 1996).

The second synthetic receptor-selective retinoid ligand is adapalene (fig. 2), a new highly stable naphthoic acid arotinoid with lipophilic properties. It does not bind to CRABP, although it enhances its synthesis, and its rank order of retinoid receptor affinity appears to be $RAR\beta > RAR\gamma \gg RAR\alpha$ (Bernard, 1993; Griffith *et al.*, 1993; Shalita *et al.*, 1996).

Future generations of such receptor subtype-selective retinoids may provide clinicians with more specific and less toxic drugs for dermatological therapy. These arotinoids, which first were introduced for the treatment of skin diseases, also may have potential as anticancer drugs (Tsambaos and Orfanos, 1982, 1983; Dreno, 1993; Orfanos *et al.*, 1997; Duvic *et al.*, 1997).

C. Future Directions

The steadily increasing knowledge concerning ligand-receptor interaction and the metabolism and molecular actions of retinoids portends new approaches for managing dermatological diseases through pharmacological modulation of the retinoid metabolic pathway. The future of retinoid therapy of these disorders seems to be moving in two directions.

First, the development of drugs that modulate retinoid metabolism by interacting with retinoid-metabolizing enzymes and/or binding proteins, and second, more retinoid receptor subtype-specific synthetic retinoid derivatives.

Drugs from the first category may permit reduction of the amount of the agent administered, thereby increasing therapeutic benefit and reducing the toxic side effects of treatment. The efficacy of the azole derivative liarazole as an inhibitor of RA 4-hydroxylase for the treatment of psoriasis in combination with RA demonstrates the usefulness of this approach. Investigation is underway to evaluate the clinical significance of our *in vitro* findings of increased intracellular RA levels after treatment with vitamin D₃ or its synthetic derivative tacalcitol (Jugert *et al.*, 1997).

It is important to emphasize that retinoids are also very effective drugs for preventing or treating cancer (Lippman *et al.*, 1997), especially skin malignancies, which present the most frequent type of human cancer (Khuri *et al.*, 1997). New retinoid regimens could lead to innovative therapy options in cutaneous cancers.

Drugs from the second category are selective retinoid receptor agonists/antagonists. As discussed above, they could offer more specific approaches by targeting specific retinoid receptors uniquely relevant for the treatment of specific skin disorders. Furthermore, this approach could reduce the occurrence of retinoid side effects.

The experimental systems used for study are crucial for defining retinoid action in the skin. For example, retinoids were reported to display effects in cultured keratinocytes that were opposite to those *in vivo* (for

review see Fisher and Voorhees, 1996). Monolayer in vitro systems exhibit responses to retinoid treatment that differ from the in vivo situation. Using keratinocytes grown on a dermal substrate without direct contact with culture medium has helped to solve this dilemma (Asselineau, 1989).

The knowledge concerning the molecular action of retinoids in the skin has increased dramatically, but the majority of steps of retinoid metabolism especially retinoid inactivation still are not fully understood. The interaction of retinoids as the central agent with other drugs represents a new dimension of dermatological therapy providing us with more specific and less toxic therapy approaches to influence cell proliferation and differentiation. Perhaps in no other area of pharmacology is the concept of using drug-drug interactions as a rationale for therapy more advanced than with retinoids in dermatology. It is likely that this strategy will prove useful in other areas as well.

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