Cytochrome P450: A Target for Drug Development for Skin Diseases

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Enzymes of the cytochrome P450 (P450 or CYP) super family are the most versatile and important class of drug-metabolizing enzymes that are induced in mammalian skin in response to xenobiotic exposure. At the same time, CYP have numerous important roles in endogenous and exogenous substrate metabolism in the skin. For example, they participate in the metabolism of therapeutic drugs, fatty acids, eicosonoids, sterols, steroids, vitamin A, and vitamin D, to name a few. In addition, in some skin diseases, for example in psoriasis, many CYP are elevated. CYP are the target of special interest in the development of drugs for skin diseases because most, if not all, drugs available in the armamentarium of the dermatologists are either substrate, inducer, or inhibitor of this enzyme family. The functional significance of drug metabolism in skin and the implication of CYP in skin pathology and therapy is an area for future investigation. A detailed insight into the mechanism of action of various cutaneous CYP, being capable of modulating the drug bioavailability, will be helpful in the development of better strategies for novel therapy against constantly increasing skin disorders. This brief review discusses some of these perspectives and suggests additional work in this research area with regard to the expression and modulation of CYP in mammalian skin as well as their implication in dermatological disorders and the therapy of skin diseases.

Keywords: Cytochrome P540/skin/drug metabolism/drug development/vitamin D/retinoic acid
J Invest Dermatol 123:417–425, 2004

Skin is the largest organ of the body that is exposed, both acutely and chronically, to a variety of chemicals either intentionally through drugs and cosmetics, or unintentionally as a result of exposure to environmental pollutants such as industrial chemicals and pesticides. This culminates into a variety of skin-related occupational health problems. For this reason, the National Institute of Occupational Safety and Health has categorized the skin diseases as one of the most pervasive occupational health problems in the USA (Rice and Cohen, 1996). In response to this issue, one major focus of health-care professionals and researchers is to design mechanism-based approaches aimed at the prevention and cure for this chronic problem. For this reason, a proper understanding of the mechanisms involved in the biotransformation of xenobiotics by the skin is of utmost importance. The xenobiotics that come in contact with the skin are biotransformed into harmless or less harmful agents by enzymatic reactions catalyzed by a variety of metabolizing enzymes present in the skin. Cytochrome P450 (P450 or CYP), the key metabolic enzyme family, is the terminal oxidase of the mixed function oxygenase system capable of metabolizing drugs and chemicals in hepatic and extrahepatic tissues including skin (Gonzales, 1989). Humans have 57 functional CYP genes and 46 pseudogenes (http://drnelson.utmem.edu/human.genecount.html). Ironically, certain xenobiotics which themselves are not carcinogenic are biotransformed by cutaneous CYP into proximate or ultimate carcinogens (Mukhtar *et al*, 1991 and references therein).

The special interest for CYP enzymes in skin is evident by the fact that most, if not all, drugs used by the practicing dermatologist are either substrate or inducer, or inhibitor of this enzyme family (Table I). It is important to mention here that CYP enzymes act on many endogenous substrates including vitamin D and vitamin A, which are widely used in clinical practice for treating a variety of dermatological disorders. Also, many ingredients in cosmetics, toiletries, and health-care products, as well as a number of allergens, toxicants, and carcinogens to which the skin is exposed, serve as substrates for CYP. In this review, we present a brief account of the research of cutaneous CYP with emphasis on its role in drug development for skin diseases.

Abbreviations: AA, arachidonic acid; AHH, aryl hydrocarbon hydroxylase; BaP, benzo(a)pyrene; CYP, cytochrome P450; CYP scc, CYP side chain cleavage; 125D,1,25-dihydroxyvitamin D(3); ECOD, 7-ethoxycoumarin deethylase; EROD, 7-ethoxyresorufin deethylase; LT, leukotrienes; β-NF, β-naphthoflavone; PAH, polycyclic aromatic hydrocarbons; RA, retinoic acid; UV, ultraviolet

CYP: A Historical Outlook

The first known prokaryotes were autotrophic and anaerobic in nature, and it is believed that the photosynthetic bacteria existed about 3500 million years ago. The photosystem-I, used by the anaerobic photosynthetic bacteria, was not capable of producing oxygen (O_2) and therefore they depended on hydrogen sulfide (H_2S), hydrogen (H_2), and/or organic molecules for electron donation. On the

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Table I. Substrates, inhibitors, and inducers of cytochrome P450 used for skin diseases[a]

Substrates	Inhibitors	Inducers
Antihistamines	Clotrimazole	Cyclosporin
Chloroquine	Griseofulvin	Dexamethasone
Crude coal tar	Itraconazole	Crude coal tar
Cyclosporin	Ketoconazole	Psoralens
Dapsone	Liarazole	retinoid acid
Glucocorticoids	Miconazole	Rifampicin
Griseofulvin	Psoralens	Ultraviolet light
Hydroxychloroquine	Retinoic acid	
Imidazoles	Voriconazole	
Psoralens		
Retinoic acid		
Steroids		
Vitamin D		

[a]This list, by no means, is complete.

other hand, the photosystem-II, which existed in Archean cyanobacteria, used water as electron donor and is believed to be the source of today's oxygen. About 3000 million years ago, our planet got rusted because of the oxidation of sulfides, iron, and organics. At this time, the prokaryotes were successfully adapting for aerobic life. A massive increase in the atmospheric oxygen occurred about 2200 million years ago that resulted in the evolution of symbiotic eucaryotes as well as the enzyme CYP, capable of detoxifying the atmospheric oxygen. Finally, as a result of limited mutations, the modern CYP evolved in the living system (Nebert and Feyereisen, 1994). The first experimental evidence of the discovery of CYP dates back to the year 1955, when an enzyme system capable of oxidizing xenobiotic compounds was identified in the endoplasmic reticulum of the liver (Axelrod 1955; Brodie *et al*, 1955). In the year 1958, two independent studies (Garfinkel, 1958; Klingenberg, 1958) detected a carbon monoxide (CO)-binding pigment in liver microsomes, which had an absorption maximum at 450 nm. This was demonstrated to be a hemoprotein of the b-type class in 1964 (Omura and Sato, 1964a, b), which was given its classical name after the strong feature in its absorption spectrum.

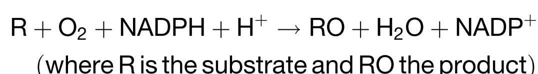
CYP are categorized based upon their amino acid sequence similarities and are grouped in families, which are made up of subfamilies. The cytochromes, P450, are named with the root CYP followed by a number designating the family, a capital letter that belongs to the subfamily and a number for the individual form. Thus, CYP1A1 denotes family "1", subfamily "A" and "1". The genes are also designated in a similar fashion, but in italics: *CYP1A1.*

CYP: General Concepts

"CYP" describes the members of an enzyme superfamily that catalyzes the oxidative biotransformation of more harmful lipophilic substrates to less harmful or harmless hydrophilic metabolites for their ultimate removal from the living system. CYP are mostly located in the endoplasmic reticulum, and to some extent in mitochondrial fractions of hepatic and extra-hepatic tissues, including skin. Structurally, CYP are heme-containing proteins consisting of iron in its +3 oxidation state. The Fe^{3+}, on reduction, is converted to Fe^{2+} that facilitates the binding of CYP with ligands such as O_2 and CO. The complex between Fe^{2+} (of P450) and CO absorbs at 450 nm and this is the reason for the classical name, "CYP".

The first experimental evidence for the existence of CYP proteins was obtained in 1958 when this pigment was characterized in pig and rat liver microsomes (Mukhtar *et al*, 1991 and references therein). Since then, a considerable amount of work has been done in defining the role of these enzymes and their functions and at present the human genome is known to encode 57 CYP proteins. The majority of CYP are involved in the metabolism of steroids, bile acids, fatty acids, eicosanoids, and fat soluble vitamins. About 15 P450 are known to be involved in the metabolism of drugs and other xenobiotic chemicals and have received attention for drug development. It is clear now that no enzyme of this family possesses a unique substrate specificity and may perform more than one catalytic function on more than one substrates. Similarly, multiple CYP may act on one chemical or substrate.

The classical reaction catalyzed by CYP is "monooxygenation", where one atom of oxygen (of O_2) is incorporated into a substrate whereas the other oxygen atom is reduced to water using an electron from NADPH as follows:



$$R + O_2 + NADPH + H^+ \rightarrow RO + H_2O + NADP^+$$
$$(\text{where R is the substrate and RO the product})$$

The metabolism of drug, in general, consists of two phases defined as phase-I and phase-II. Phase-I includes the reactions resulting in the incorporation of a polar reactive group into the inert molecule which, in turn, becomes the substrate for phase-II enzymes.

In general, CYP-dependent reactions result in detoxification of a pharmacologically active drug into its inactive forms, but a number of examples also exist where the inert carcinogenic agents, generally referred to as "precarcinogens", are metabolized into proximate or ultimate carcinogens by the CYP system. Benzo(a)pyrene (BaP), present in crude coal tar widely used in dermatology and also generated by the incomplete combustion of fossil fuel, a ubiquitous pollutant, is an excellent example of this type of reaction (Conney, 1982). The metabolic products formed in phase-I undergo conjugation reactions with glucuronide, sulfate, glutathione, etc. in phase-II metabolism. The product of this reaction is generally hydrophilic and is readily excreted out from the body.

The CYP, in addition to the hydroxylation reactions, also oxidize the heteroatoms including nitrogen and sulfur. The CYP-mediated oxidation of aliphatic double bonds or

aromatic hydrocarbons leads to the formation of epoxides, which may either be labile intermediates or stable products. These epoxides can then be converted into dihydroxy metabolites known as "diols", by the hydrolysis reactions either non-enzymatically using water or by an enzymatic reaction catalyzed by epoxide hydrolase (Conney, 1982).

Drug Metabolism in Skin

The barrier function of the skin is evident from its ability to metabolize the agents, which diffuse through it, leading to their altered biological activities. The xenobiotics, to which the skin is exposed, and the topically applied drugs may undergo a degradation or activation process, which may result in skin sensitization or even carcinogenesis. The CYP are the most important among the drug-metabolizing enzymes in the skin, by virtue of their crucial role in controlling the steady-state concentrations of a variety of bioactive substances including fatty acids, steroids, prostaglandins, glucocorticoids, retinoids, and leucotrienes (LT) (Mukhtar and Khan, 1989; and references therein). Being capable of modulating the drug bioavailability in the skin, the CYP are important targets for the development of better strategies for the therapy of skin disorders.

Skin is a heterogenous tissue consisting of several structural proteins and therefore resistant to the conventional preparative processes, and the enzyme activities are also much lower in skin than in most other tissues. In 1953, Norden showed that following cutaneous application of benzopyrene to guinea-pigs, a *metabolic fluorescence* appeared in the epidermis, hair follicles, and sebaceous glands of the skin, suggesting the occurrence of metabolism in the skin (Mukhtar *et al*, 1991; and references therein). Since then, a considerable body of work, from many laboratories including ours, has been done in the area of CYP and drug metabolism in the skin (Mukhtar *et al*, 1991; and references therein).

RT-PCR, immunoblot, immunohistochemistry, and catalytic assays revealed that proliferating normal human skin keratinocytes show the expression of various CYP enzymes, especially CYP1A1, CYP1B1, CYP2B6, CYP2E1, and CYP3A. In comparison with other cells present in human skin, e.g., monocytes (Baron *et al*, 2001 and references therein), lymphocytes (Baron *et al*, 2001 and references therein), and fibroblasts (not published), considerably higher amounts of these enzymes were found to be present in keratinocytes. By immunofluorescent techniques, CYP1A1, CYP2B6, CYP2E1, and CYP3A were found to be expressed dominantly in keratinocytes compared with fibroblasts (Baron *et al*, 2001 and references therein). Among various CYP, CYP1A1 is the best conserved of all the xenobiotic metabolizing enzymes and is one of the few CYP that are known to be expressed in skin. The constitutive levels of CYP1A1 in skin are too low to be measured without an exposure to some exogenous inducer. CYP1A1, despite of being well conserved throughout the animal kingdom, is not known to possess any unique endogenous substrate. The various polycyclic aromatic hydrocarbons (PAH), such as BaP, 3-methylcholanthrene, or 7,12-dimethylbenz(a)anthracene, are excellent substrates for CYP1A1. The CYP1A1-mediated reactions result in mutagenic and carcinogenic metabolites of the parent compounds. Several studies have demonstrated the inductibility of CYP1A1 and the related monooxygenase activities, in human and rodent skin as well as human hair follicles, following the exposure to PAH, β-naphthoflavone (β-NF), and glucocorticoid (Mukhtar and Bickers, 1983; Finnen *et al*, 1984; Merk *et al*, 1987; Whitlock, 1987). Further, the induction of CYP1A1 mRNA, in rat epidermis, and cultured human epidermal keratinocytes by benz(a)anthracene, and β-NF has been shown using a RT-linked PCR (Khan *et al*, 1992). The treatment, with these inducers, was found to result in several-fold enhancement in aryl hydrocarbon hydroxylase (AHH) activity in rat epidermis as well as in human keratinocytes. The exposure to β-NF and BaP also resulted in a significant enhancement of AHH activity in rat epidermis and in human keratinocytes (Khan *et al*, 1992). In another study, Raza *et al* (1992) demonstrated that topically applied β-NF (40 μg per kg) to rats resulted in a significant induction of CYP1A1 expression and monooxygenase activity in epidermis. Stauber *et al* (1995) demonstrated that CYP1A1 expression also depends on the progression of cell differentiation. In this study, it was shown that topical application of β-NF, to mice, resulted in $\sim$ 87-fold enhancement in epidermal 7-ethoxyresorufin deethylase (EROD) activity per cell and a manifold increase in CYP1A1 expression in the epidermis. It was also found that the β-NF application leads to a CYP1A1 increase only in 40%–50% of the isolated epidermal cells. Moreover, the population of epidermal cells containing β-NF-induced elevated CYP1A1 expression was enriched in superbasal differentiated cells and also contained some basal cells. Similarly, Reiners *et al* (1992) demonstrated the existence of a differential expression of both basal and inducible phase-I and phase-II metabolizing enzymes in the epidermis that was regulated as a function of the stage of epidermal differentiation.

Association of AHH Activity with CYP in Skin

The expression and modulation of CYP1A1 is associated with the AHH activity in the skin (Lilienblum *et al*, 1985; Pham *et al*, 1989; Gonzalez, 1995), which is responsible for the metabolic activation of PAH such as BaP. The 7-ethoxycoumarin deethylase (ECOD) and the EROD activities are also catalyzed by CYP1A1 in the skin. Wattenberg and Leong (1962) demonstrated that the application of 3-methylcholanthrene resulted in an enhanced cutaneous AHH activity in rats. Several other studies have also shown significant induction of AHH and related enzyme activities, i.e. ECOD and/or EROD in skin by the topical or systemic administration of a wide range of xenobiotics including PAH, tetrachlorodibenzo-*p*-dioxin, and Aroclor 1254. Studies have also shown that the topical application was the most effective of all modes of xenobiotics exposure in terms of the induction in the above-mentioned enzyme activities (Khan *et al*, 1989; Raza and Mukhtar, 1993). Schlede and Conney (1970) demonstrated that topically applied 3-methylcholanthene results in a 10-fold increase in cutaneous AHH activity in rats. Levin *et al* (1972) and Alvares *et al* (1973) demonstrated the induction of BaP hydroxylase in human skin by PAH. Bickers *et al* (1974, 1975) demonstrated

that a commercial mixture of polychlorinated biphenyls, Aroclor 1254, or the microscope immersion oil containing these compounds, when applied topically, results in significant AHH induction in skin. Thompson and Slaga (1976) demonstrated the induction of cutaneous AHH activity in the mouse by topical application of PAH.

Weibel *et al* (1971) demonstrated that the topical application of benz(a)anthracene results in significant AHH induction in mouse skin. Later, it was found that the AHH activity in mouse skin possesses certain features analogous with the hepatic NADPH-dependent mixed function oxidase (Weibel *et al*, 1975). Briggs and Briggs (1973) showed that the topical application of selected adrenocorticosteroids used in the therapy of certain dermatological disorders may induce AHH activity in mouse skin. These authors suggested that the inducible enzyme activity in skin may have a correlation with the therapeutic efficacy of adrenocorticosteroids in skin therapy. Mukhtar and Bickers (1981) demonstrated that the topical application of BaP or Aroclor 1254 results in the AHH induction in skin as well as other tissues. The rate of increase in the enzyme activity, however, was much greater in skin as compared with the other tissues (Mukhtar and Bickers, 1981). These studies established that even though the specific enzyme activities are much lower in the skin as compared with liver, they may make a major contribution in the overall metabolism of xenobiotics in the body. Several other reports from our group further demonstrated the induction of cutaneous AHH, ECOD, and EROD activities in animal models, by a number of agents generally used in the therapy of several skin conditions (Bickers *et al*, 1982; Mukhtar and Bickers, 1982; Mukhtar *et al*, 1982; Merk *et al*, 1989).

In a recent study, Harris *et al* (2002) measured the activity of CYP1A1, i.e., EROD and seven ECOD activities. The enzyme activities were determined in cultured keratinocytes, reconstructed epidermal models, and samples of human epidermis or hair follicle. The data demonstrated that EROD activity was detectable in cultured keratinocytes and was induced by 3-MC and β-NF. Induced EROD activity was found to be inhibited by clotrimazole in a concentration-dependent manner. EROD activity was not detected in either hair follicles or untreated epidermal models but was found to be induced by 3-MC, and clotrimazole was able to inhibit the induced EROD activity. This study suggested that reconstructed skin models may be useful to study the effects of non-water-soluble topical formulations on xenobiotic metabolism (Harris *et al*, 2002).

Other Members of the CYP Enzyme Family

Many studies have implicated the existence of multiple CYP in mouse and human skin. In a study from our laboratory (Jugert *et al*, 1994), we demonstrated that a topical application of dexamethasone to mice results in a significant induction of EROD, 7-pentoxyresorufin-*O*-depentylase, *p*-nitrophenol hydroxylase, and erythromycin *N*-demethylase activities, which are the monooxygenases catalyzed preferentially by CYP1A1, CYP2B1, CYP3E1, and CYP3A, respectively. The immunoblot analysis of cutaneous microsomes, using antibodies against CYP1A1, CYP2B1, CYP2E1, and CYP3A, showed that the topical application of dexamethasome resulted in increased immunoreactivity (2–10-fold) for these CYP. It was also found that the constitutive mRNA expression for CYP1A1 and CYP2E1 remains unaltered by dexamethasone treatment.

Another study from our laboratory reported the induction of EROD, 7-pentoxyresorufin-*O*-depentlylase, and erythromycin *N*-demethylase activities in mouse skin by the topical application of pyridine, an amphipathic solvent widely used in industries and a constituent of tobacco and its smoke (Agarwal *et al*, 1994). The treatment of skin with pyridine resulted in an enhanced reactivity with the antibodies directed against CYP1A1, CYP2B1, and CYP3A.

Sutter *et al* (1994) demonstrated a 100-fold increase in the level of CYP1B1 mRNA following treatment with nanomolar concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in primary cultures of normal human epidermal keratinocytes. The CYP1B1 has been shown to play a major role in the metabolism of PAH such as 7,12-dimethylbenz(a)anthracene, a known skin carcinogen.

In a recent study, the complement and level of expression of CYP enzymes in male Fischer F344 rat whole skin and cultured keratinocytes were investigated using a panel of mono-specific antibodies (Zhu *et al*, 2002). In whole skin microsomal fraction, immunoreactive bands corresponding to CYP2B12, CYP2C13, CYP2D1, CYP2D4, CYP2E1, CYP3A1, and CYP3A2 were detected whereas CYP1A1, CYP1A2, and CYP2C12 were found to be absent. In this study, the keratinocytes were isolated from rat skin and changes in the levels of CYP3A1, CYP3A2, and CYP2E1 determined. Levels were found to be low in fresh isolated keratinocytes, but they increased markedly in culture, reaching a maximum at 10–14 d, where they were similar to those found in fresh skin. This study suggested that primary keratinocytes grown in culture for 10–14 d may provide a useful experimental model to study CYP-catalyzed metabolism of xenobiotics in skin.

Thiboutot *et al* (2003) studied the steroidogenic pathway upstream from dehydroepiandrosterone by assessing the presence of members of the CYP side chain cleavage (CYP scc) system. This system catalyzes the initial step in steroid hormone synthesis following translocation of cholesterol to the inner mitochondrial membrane. An SV40-immortalized human sebaceous gland cell line (SEB-1) was established for investigating the CYP scc system. In this study, the presence of CYP scc, adrenodoxin reductase, CYP c17, and steroidogenic factor 1 was documented in human facial skin, human sebocytes, and SEB-1 sebocytes. Using immunohistochemistry, antibodies to the above proteins were localized to epidermis, hair follicles, sebaceous ducts, and sebaceous glands in sections of facial skin. Biochemical activity of CYP scc and CYP c17 was demonstrated in SEB-1 sebocytes using radioimmunoassay. Gene array expression analysis and quantitative PCR indicated that mRNA for CYP scc is more abundant than mRNA for both CYP c17 and steroidogenic factor 1 in sebaceous glands and SEB-1 cells. These data demonstrated that the skin is in fact a steroidogenic tissue (Thiboutot *et al*, 2003). The clinical significance of this finding in mediating androgenic skin disorders such as acne, hirsutism, or androgenetic alopecia, however, remains to be established.

In a recent study, Yengi *et al* (2003) developed real-time PCR assays to quantitate levels of CYP mRNA in human tissues. The assay was used to quantitate CYP mRNA levels in human skin samples from 27 healthy volunteers. The major enzymes detected were CYP1B1, CYP2B6, CYP2D6, and CYP3A4 with mean values of 2.5, 2.6, 2.7, and 1.1 fg/18S rRNA in 50 ng total RNA, respectively. Lower levels of CYP2C18, CYP2C19, and CYP3A5 were also detected whereas CYP1A2, CYP2A6, and CYP2C8 were below limits of detection. There was inter-individual variation in the levels of mRNA among the 27 subjects studied.

Retinoic Acid (RA) and CYP

The retinoids are widely used for treating a variety of skin disorders and because of this fact, several reports have investigated retinoid metabolism in skin. RA plays an important role in the development of the epithelial tissues (Shapiro, 1986). RA metabolizes via the hydroxylation of its cyclohexenyl moiety resulting in the formation of inactive 4-hydroxy-RA that further oxidized to keto-RA and other polar metabolites. It has been widely shown that these reactions are CYP dependent (Roberts *et al*, 1980; Williams and Napoli, 1985, 1987). Studies have shown that the *in vitro* hydroxylation of RA is catalyzed by the CYP (Roberts *et al*, 1991; Martini and Murray, 1993). Li *et al* (1995) have demonstrated that RA is a CYP1A1 substrate, and the constitutive expression of CYP1A1 mRNA and protein are significantly downregulated following the topical application of RA on human skin. It was also found that RA application leads to the downregulation of CYP1A2 mRNA by 93%. Liarozole, which is an imidazole-containing compound, is known to inhibit the CYP-mediated metabolism of t-RA resulting in an increase of the retinoid in skin and plasma (VanWauwe and Janssen, 1989; Dockx *et al*, 1995). Based on this finding, it was suggested that this agent may be used as an anti-psoriatic drug. In another study, Stoppie *et al* (2000) demonstrated that an oral administration of R115866 (2.5 mg per kg), an inhibitor of the CYP-mediated metabolism of RA, to rats induced marked and transient increases of endogenous RA levels in several tissues including skin. This study suggested that CYP26 is capable of metabolizing RA in the skin. In fact, CYP26 was cloned and characterized in zebra fish, human, and mouse tissues in 1996 (White *et al*, 1996). CYP26 displayed specificity toward RA and was shown to function as an important regulator of differentiation and a possible modulator of disease states by controlling retinoid concentration and homeostasis (Haque and Anreola, 1998). Three members of this CYP family are known to date. The CYP26A1 and CYP26B1 were shown to be responsible for catabolism of RA both in the embryo and the adult (Taimi *et al*, 2004 and references therein). In a very recent study, the identification, molecular cloning, and substrate characterization of a third member of the CYP26 family, named CYP26C1, was described (Taimi *et al*, 2004). This study suggested that although CYP26C1 shares extensive sequence similarity with CYP26A1 and CYP26B1, its catalytic activity appears to be distinct from those of other CYP26 family members (Taimi *et al*, 2004). It was suggested that CYP26C1 can also recognize and metabolize 9-cis-RA and is much less sensitive than the other CYP26 family members to the inhibitory effects of ketoconazole (Taimi *et al*, 2004).

Vitamin D and CYP

Studies have demonstrated that vitamin D(3) is a natural product of a sunlight-mediated process in the skin and its biological function is dependent on specific CYP enzymes (Omdahl *et al*, 2003). CYP27B1 is the regulatory rate-limiting enzyme that controls the bioactivation process and the resultant 1,25-dihydroxyvitamin D(3) (1,25D) is biologically active that directs the multitude of vitamin D-dependent actions involved with calcium homeostasis, cellular differentiation and growth, and the immune responses. The circulating and cellular level of 1,25D is governed through a coordinated process that involves the hormone's synthesis and degradation. Central to the degradation and turnover of 1,25D is the regulatory multi-catalytic CYP24 enzyme that directs the introduction of C-24R groups into targeted 25-hydroxy substrates (Omdahl *et al*, 2003). Thus, Vitamin D and its analogs possess a potential for novel drug development for skin diseases.

In a study, Kang *et al* (1997) keratomed normal adult human skin after a 2-d exposure to 1,25D, 9-c-RA, t-RA, and ketoconazole. 1,25D was found to cause a concentration-dependent increase in CYP24 mRNA expression. The activity of epidermal 24-OHase was also induced by 1,25D. Compared with vehicle, neither of the RA isomers nor ketoconazole alone induced CYP24 mRNA. Further, an addition of 9-c-RA or t-RA to 1,25D, resulted in a synergistic increase in CYP24 mRNA. Similarly, 1,25D along with ketoconazole resulted in an increased 24-OHase mRNA, in a synergistic fashion. Ketoconazole inhibited *ex vivo* 1,25D-induced epidermal CYP24 activity (Kang *et al*, 1997). This study showed that (i) CYP24 mRNA induction is a sensitive reporter of 1,25D activity *in vivo*, (ii) retinoid X receptor (RXR) bound to vitamin D receptor (VDR) is not a silent partner *in vivo*, because 9-c-RA enhances 1,25D-liganded RXR/VDR stimulation of the 1,25D-responsive enhancer element (VDRE) containing CYP24 gene, and (iii) ketoconazole inhibition of CYP24 enhances 1,25D activity by impeding its breakdown (Kang *et al*, 1997). Based on this work, the authors suggested that the synergistic response of human skin to topical 1,25D and/or 1,25D analogs plus RXR retinoids and/or ketoconazole may be exploited to give a desired therapeutic response with less 1,25D, thereby minimizing the potential calcemic risk from systemic absorption of 1,25D (Kang *et al*, 1997). A detailed account on CYP24 can be appreciated from a recent review by Schuster *et al* (2003 and references therein).

Based on several studies, it is now clear that the active form of vitamin D and its analogs suppress growth and stimulate the terminal differentiation of keratinocytes (Kira *et al*, 2003). It is also known that in psoriatic lesions, epidermal keratinocytes exhibit hyper-proliferation and impaired differentiation triggered by inflammation. Therefore, it is quite reasonable to assume that vitamin D is effective on psoriasis. In recent years, analogs of vitamin D3 have been used as topical therapy for psoriasis. This topic is well

reviewed by Kira *et al* (2003). Several studies have shown that treatment of skin diseases including psoriasis, with vitamin D or its analogs, however, is associated with side effects, irritant contact dermatitis being the main adverse effect with vitamin D analogs (Bruner *et al*, 2003).

Importance of CYP in Drug Development for Skin Diseases

Because of the critical role of CYP in the biochemistry of fungi and protozoa, these enzymes may serve as targets for drug development for certain types of skin disorders (Cauwenbergh, 1986). Several CYP inhibitors including some anti-fungal agents have successfully been used in therapy against skin disorders. The fungicidal and the fungistatic properties of drugs depend on their ability to (i) inhibit the synthesis of ergosterol, a major constituent of the fungal cell membrane, and (ii) block CYP-dependent demethylation of lanosterol. The imidazoles, for example, miconazole and clotrimazole, are generally administered topically whereas the ketoconazoles and itraconazole possess a higher activity when administered systemically (Vecchini and Michel, 1994; and references therein).

The mammalian CYP also play important roles in the biosynthesis of skin endobiotics such as LT and RA (Holtzman *et al*, 1989; Mukhtar *et al*, 1989; Vanden Bossche and Willemsen, 1991), which are shown to have implications in the development of certain skin disorders like psoriasis and atopic eczema (Voorhees, 1983). It is important to mention here that, in skin, the molecular events, which drive migration of polymorphonuclear leukocytes (PMN), are triggered by the local release of a variety of chemotactic and stimulatory factors (Marleau *et al*, 1999 and references therein). These factors include chemokines, cytokines, complement-derived protein fragments, lipid mediators, and bacterial products, such as formylated peptides that coordinate cytoskeletal rearrangements and adhesive changes essential for effective cell motility (Marleau *et al*, 1999 and references therein). Invading PMN are themselves a rich source of bioactive lipids, including oxygenated derivatives of arachidonic acid (AA) that are generated through the 5-lipoxygenase (5-LO) pathway and that may serve as both intracellular and extracellular mediators (Marleau *et al*, 1999 and references therein). A well-investigated group of these AA derivatives are the LT. In support of the important role of LTB4 in inflammation, inhibitors of 5-LO product biosynthesis and LTB4 receptor antagonists have proved to be beneficial in different experimental models of inflammation and in some pathologic conditions (Marleau *et al*, 1999 and references therein). LTB4 is a highly potent leukocyte chemotactic compound, which has been identified in chamber fluid and scale from psoriatic skin lesions (Marleau *et al*, 1999 and references therein). The ability of LTB4 to reproduce the inflammatory events of psoriasis, by topical application to the skin of normal human volunteers, was studied by Camp *et al* (1984). Persistent visible inflammatory reactions were elicited by application of LTB4. This study provides evidence for the role of LTB4 in the pathogenesis of psoriasis (Camp *et al*, 1984).

Wong *et al* (1986) topically applied clobetasol propionate or vehicle ointment to psoriatic plaques on patients. Skin chamber exudates from untreated, steroid-, and vehicle-treated lesions were assayed for AA, LTB4, prostaglandin E2 (PGE2), and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE). Significant reductions in AA and LTB4 were observed in steroid-treated lesions (Wong *et al*, 1986). The reduction in 12-HETE levels observed after steroid treatment was not statistically significant. PGE2 levels in lesional psoriatic skin were unaltered. The reduction of AA and LTB4 was found to be associated with clinical improvement of psoriasis (Wong *et al*, 1986). Fogh *et al* (1989) demonstrated that the inflammatory mediators PGE2 and LTB4 are present in lesional skin of atopic dermatitis (AD) subjects in biologically active concentrations. The authors suggested that because these mediators are able to induce cutaneous inflammation and to modulate cellular immunity, they may be involved in the biochemical processes leading to AD (Fogh *et al*, 1989).

In view of the involvement of CYP in LT and RA metabolism, some azole derivatives (such as imidazole) have been shown to possess anti-psoriatic action (Farr *et al*, 1985). The imidazoles are proved to be more successful in the treatment of certain androgenic skin lesions such as hirsutism, alopecia, and acne (Carvalho *et al*, 1985; Ghetti *et al*, 1986; Rottein *et al*, 1992). The retinoids have extensively been used for the treatment of various acne types (Darmon, 1991). Studies from our laboratory have also shown that several CYP inhibitors such as synthetic flavones and plant phenols reduce the amount of carcinogenic compounds in skin by inhibiting the AHH activity which, in turn, decreases the binding of carcinogens to DNA (Das *et al*, 1987). Other studies from our laboratory and elsewhere have also shown the therapeutic potential of ketoconazole, clotrimazole, and liarozole against a number of dermatological lesions, including cancer (Mukhtar *et al*, 1984; Das *et al*, 1986; DeCoster *et al*, 1986).

Kuijpers *et al* (1998), as part of a large, double-blind, randomized clinical study, investigated the cell biological alterations in uninvolved and lesional skin of 20 patients with severe plaque psoriasis, who were treated with liarozole. The extent and severity of the skin lesions, as recorded by the Psoriasis Area and Severity Index score, was significantly reduced after treatment by liarozole. A significant decrease in the markers for inflammation (neutrophils), epidermal proliferation (Ki-67-positive cells), normal differentiation (transglutaminase), and abnormal differentiation (cytokeratin 16 and skin-derived anti-leucoproteinase; SKALP) was seen (Kuijpers *et al*, 1998). SKALP levels in serum showed a statistically significant correlation with clinical scores. This study concluded that liarozole is an effective anti-psoriatic agent (Kuijpers *et al*, 1998). Bhushan *et al* (2001) studied the effectiveness of oral liarozole in the treatment of palmoplantar pustular psoriasis. This study suggested that liarozole is an effective and well-tolerated therapy for palmoplantar pustular psoriasis (Bhushan *et al*, 2001).

Similarly, ketoconazole is used to treat several skin conditions such as infections caused by a fungus or yeast. Ketoconazole creams are used to treat athlete's foot (tinea pedis; ringworm of the foot), ringworm of the body (tinea

corporis), ringworm of the groin (tinea cruris; jock itch), seborrheic dermatitis, sun fungus (tinea versicolor; pityriasis versicolor), and Yeast infection of the skin (cutaneous candidiasis). Ketoconazole binds to CYP enzymes and inhibits cells from producing ergosterol, the main component of the cell wall. Both liarozole and ketoconazole, as well as certain other azoles, are CYP-based drugs that have shown great promise for several dermatological conditions.

Several azole-based inhibitors of CYP have been shown to be useful for a variety of dermatological conditions. Voriconazole is a broad-spectrum azole anti-fungal agent structurally derived from fluconazole. It is indicated for the treatment of invasive aspergillosis and serious fungal infections caused by *Scedosporium apiospermum* and *Fusarium* species in patients who are unable to tolerate or are refractory to other anti-fungal therapy (Jeu *et al*, 2003 and references therein).

Association of CYP with Ultraviolet (UV) Exposure and Drug Development for Psoriasis

Psoriasis is a disease that has long been a challenge for dermatologists. The complexity of treating psoriasis may be attributed to both the chronic and persistent nature of this disease, as well as to the numerous available therapies. The choice of the appropriate treatment must take into account many factors, including the characteristics and locations of the lesions and extent of involvement. Studies have shown that there are unpredictable inter-individual differences in response to UV radiation, used in the treatment of psoriasis and other common skin diseases. It is therefore necessary to define the phenotypic markers that correlate with individual treatment outcomes. In two recent studies, Smith *et al* (2003a, b) used quantitative real-time RT-PCR to investigate inter-individual differences in the cutaneous expression of CYP, and investigated the regulation of gene expression by UV radiation and in lesional psoriatic skin *versus* normal individuals. In psoriatic plaque, two newly identified members of CYP family, CYP2S1 and CYP2E1, were found to be significantly enhanced, implying a differential adaptive response to oxidant exposure in lesional psoriatic skin. UV exposure also induced the expression of these enzymes. Considerable inter-individual variation was found, indicating that these genes may be associated with individuality in response to UV radiation (Smith *et al*, 2003a, b).

In a study from this laboratory, Katiyar *et al* (2000), using immunohistochemistry, RTPCR, and western blot analysis, investigated the cellular distribution and localization of CYP1A1 and CYP1B1 in human skin, and their induction by UVB exposure. CYP1A1 was found to be primarily localized in the basal cell layer of the epidermis in non-UVB-exposed skin, whereas CYP1B1 was localized in the epidermal cells other than the basal cell layer. The authors suggested that the localization of CYP1A1 and CYP1B1 in human skin are different and may be related to keratinocyte differentiation. Further, UVB exposure to solar-ultraviolet-protected skin (buttock site) was found to result in an UVB concentration-dependent (0–4 minimal erythema doses) and time-dependent (0–48 h) induction of both CYP1A1 and CYP1B1 in the epidermis. It was suggested that UVB-mediated induction of both CYP1A1 and CYP1B1 in human skin may result in enhanced bioactivation of PAH and other environmental pollutants to which humans are exposed, which could make the human skin more susceptible to UVB-induced skin cancers or allergic and irritant contact dermatitis.

Gonzalez *et al* (2001) tested the effects of UVR, a source of oxidative stress, on the expression of mRNA coding for several CYP isoforms, with particular reference to the CYP2E1 and CYP4A11 isoforms, which might play a role in lipid metabolism in human keratinocytes. The data demonstrated that mRNA for CYP2E1, CYP1A1, and CYP3A5 were expressed in all the keratinocyte preparation tested; however, neither CYP3A4 nor CYP3A7 was detected, either in the presence or absence of UVR treatment. CYP19Aro, CYP2C19, and CYP26 were not expressed constitutively, although some induction of CYP19Aro was seen after combined UVB and UVA irradiation. CYP4A11 mRNA was not detected in any keratinocyte preparation either under control conditions or after UVB treatment. In non-irradiated keratinocyte microsomes, CYP4A11 protein was found to be expressed and UVA treatment of the keratinocytes induced CYP4A11 mRNA expression after 24 h. The authors suggested that CYP4A11 may participate in the defense mechanism against UVA-induced oxidative damage (Gonzalez *et al*, 2001).

Conclusion

Thus, CYP research is accepted as an integral part of drug development for skin diseases and skin-care products as well. Work leading to this point includes biochemical studies on CYP in experimental animal models and application to human systems. Because of the diversified functions of CYP enzyme family, Coon *et al* (1996) suggested a new name, "diversozyme", for this class of enzymes. The CYP have a very vast range of substrates, including health- and beauty-care products, industrial pollutants, anesthetics, solvents, dyes, and plant products (such as flavones and odorants), to which the human skin is either intentionally or un-intentionally exposed. The mechanism of action and regulation of CYP could therefore be important for devising novel strategies for the management of a variety of cutaneous disorders. Therefore, additional research in this important area is needed. The efficacy of therapeutic agents, which is dependent on skin metabolism, may be modulated by CYP and therefore a search for novel CYP, their substrates in the skin and understanding the mechanism of their action is required. This may be helpful in designing newer approaches for the management of skin disorders.

Further, in dermatology clinics, several drugs are used whose efficacy and toxicity is not well studied. This puts the patients on risk of possible side effects without the benefit of therapeutic response. It is also not possible to predict certain rare but potentially life-threatening adverse reactions such as toxic epidermal necrolysis and Stevens–Johnson syndrome. In order to optimize patient management, it is absolutely essential that we are able to identify those at risk of toxicity and those who are therapy

resistant. One potential solution to these problems is the application of pharmacogenetics and pharmacogenomics to clinical practice. These approaches will change not only the way drugs are selected but will be important for designing clinical trials. In fact, gene polymorphisms are the basis of this inter-individual response to drug therapy and they determine individual absorption, disposition, metabolism, and excretion of drugs. They also determine the sensitivity of drug target sites, such as receptors. The best-characterized pharmacogenetic polymorphisms are those of the CYP family, for which genetic tests are already available for the CYP2D6 gene. The identification of susceptibility genes for common diseases with polygenic inheritance patterns may also provide information on genetic polymorphisms resulting in variation in drug response. Thus, pharmacogenetic knowledge may be an important tool for rational drug design for dermatological disorders and for other diseases as well.

DOI: 10.1111/j.0022-202X.2004.23307.x

Manuscript received November 11, 2003; revised April 9, 2004; accepted for publication May 4, 2004

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