Activators of the Nuclear Hormone Receptors PPARα and FXR Accelerate the Development of the Fetal Epidermal Permeability Barrier

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Abstract

Members of the superfamily of nuclear hormone receptors which are obligate heterodimeric partners of the retinoid X receptor may be important in epidermal development. Here, we examined the effects of activators of the receptors for vitamin D₃ and retinoids, and of the peroxisome proliferator activated receptors (PPARs) and the farnesoid X-activated receptor (FXR), on the development of the fetal epidermal barrier in vitro. Skin explants from gestational day 17 rats (term is 22 d) are unstratified and lack a stratum corneum barrier in vitro. Skin explants from gestational day 17 rats, which are obligate heterodimeric partners of the retinoid X receptor, on the development of the fetal epidermal barrier in vitro. Skin explants from gestational day 17 rats (term is 22 d) are unstratified and lack a stratum corneum (SC). After incubation in hormone-free media for 3–4 d, a multilayered SC replete with mature lamellar membranes in the interstices and a functionally competent barrier appear. 9-cis or all-trans retinoic acid, 1,25 dihydroxyvitamin D₃, or the PPARγ ligands prostaglandin J₂ or troglitazone did not affect the development of barrier function or epidermal morphology. In contrast, activators of the PPARα, oleic acid, linoleic acid, and clofibrate, accelerated epidermal development, resulting in mature lamellar membranes, a multilayered SC, and a competent barrier after 2 d of incubation. The FXR activators, all-trans farnesol and juvenile hormone III, also accelerated epidermal barrier development. Activities of β-glucocerebrosidase and steroid sulfatase, enzymes previously linked to barrier maturation, also increased after treatment with PPARα and FXR activators. In contrast, isoprenoids, such as nerol, cis-farnesol, or geranylgeraniol, or metabolites in the choleseterol pathway, such as mevalonate, squalene, or 25-hydroxycholesterol, did not alter barrier development. Finally, additive effects were observed in explants incubated with clofibrate and farnesol together in suboptimal concentrations which alone did not affect barrier development. These data indicate a putative physiologic role for PPARα and FXR in epidermal barrier development. (J. Clin. Invest. 1997. 100:705–712.) Key words: stratum corneum • farnesol • RXR • juvenile hormone • clofibrate

Introduction

Extracellular lipids in the stratum corneum (SC), the outermost layer of the epidermis, provide a hydrophobic seal between the organism’s interior and the xeric external environment (1, 2), a critical requirement for terrestrial life. The SC also provides the initial defense against microbial invasion, ingress of xenobiotics, UV light, and physical trauma. Because the epidermal barrier matures late in intrauterine development, i.e., during the third trimester, very premature infants lack a competent barrier, contributing to their morbidity and mortality (3–5).

Lipids are delivered to the SC by the exocytosis of lamellar bodies from granular cells, and these lipids are subsequently processed and reorganized into a continuous matrix of lamellar unit structures, characteristic of a mature SC and a competent barrier to transepidermal water loss (1, 2, 6). Our studies in fetal rats have shown, both in utero and in a skin explant model, that the formation of a competent barrier by day 21 of gestation coincides with the appearance of morphologically mature, extracellular lamellar membranes in the SC (7, 8). Several hormones, including glucorticoids, estrogen, and thyroid hormone, the actions of which are mediated by nuclear receptors, accelerate SC and barrier development in these models, and induce increased levels of lipid metabolic enzymes which are closely associated with barrier maturation (8–12).

The glucocorticoid and estrogen receptors belong to a subgroup of the nuclear receptor superfamily of transcription factors which function as ligand-induced homodimers (for review see reference 13). Another subgroup, which includes the vitamin D receptor, thyroid hormone receptor, retinoid acid receptor (RAR), and the peroxisome proliferator-activated receptors (PPARs), requires heterodimerization with the retinoid X receptor (RXR) for effective DNA binding (14, 15). Recent studies have shown that the retinoid receptors are important in epidermal development, e.g., overexpression of a dominant negative RAR mutant (α subunit) in basal cells results in a thin epidermis and dry, scaly skin (16), whereas overexpression in suprabasal cells results in the absence of extracellular SC membranes and an incompetent barrier (17, 18). These effects can be attributed to the ability of the truncated RAR to bind RXR and form transcriptionally inactive heterodimers, thereby subverting the activity of RXR and its partners.

In addition to RXR, other members of the RXR group of nuclear receptors, which may influence epidermal development, include the receptors for thyroid hormone and 1,25-dihydroxyvitamin D₃, and the PPARs (subtypes α, δ, and γ) (19–21). The PPARs are activated by a variety of fatty acids and arachidonic acid metabolites, as well as by hypolipidemic agents, such as clofibrate (19–21). The α isoform plays a role in...

1. Abbreviations used in this paper: FXR, farnesoid X-activated receptor; JH III, juvenile hormone III; PPAR, peroxisome proliferator-activated receptor; RA, retinoic acid; RAR, RA receptor; RXR, retinoid X receptor; SC, stratum corneum.

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the regulation of lipid metabolism in liver, intestine, and kidney, while the γ subtype is involved primarily in the regulation of adipocyte differentiation (19–22). The δ isoform is widely expressed, but its function is the least understood. Another newly described receptor, which is an obligate partner of the RXR, is the farnesoid X-activated receptor (FXR) (23). This receptor is activated by farnesol and juvenile hormone III (JH III) (23). JH III, like farnesol, is a metabolite of farnesyl pyrophosphate, an important intermediate in the cholesterol biosynthetic pathway. FXR is expressed in several tissues that are active in steroidogenesis, such as liver, gut, adrenal gland, and kidney (23). Since both fetal and adult epidermis are highly active sites of cholesterol and fatty acid synthesis (24, 25), it is likely that they generate a variety of endogenous activators of both FXR and PPAR.

In earlier studies, we showed that one ligand for the RXR-interacting family, thyroid hormone, accelerates fetal barrier development (8). In this study, we determined whether other ligands or activators of the RXR superfamily of nuclear receptors accelerate fetal skin maturation, using a previously described model in which epidermal development in fetal skin explants morphologically and functionally mirror the kinetics of developmental changes that occur in utero (8). We report here that activators of both FXR and PPARs accelerate the formation of a mature SC and functional epidermal permeability barrier, while ligands for the RAR, RXR, PPARγ, and vitamin D receptor had no effect.

Methods

Organ culture model and measurement of barrier function. Timed pregnant Sprague-Dawley rats (plug date = day 0) were obtained from Simonsen Laboratories (Gilroy, CA) and fetuses were delivered prematurely on day 17. Full-thickness flank skin was excised from fetal rats and incubated dermis-side down on collagen membrane inserts (3 µm pore size), as described previously (8). It was not possible to obtain intact skin from animals of an earlier gestational age. Epidermal water loss was measured gravimetrically in explants after 2 d of incubation, and reported as mg water lost/mm²/h, as previously described (8). Longer incubations were not carried out because as described previously (8) by 3 d there is variable formation of a competent barrier. Prostaglandin J3 (15-deoxy-Δ12,14-prostaglandin J3) was obtained from Cayman Chemical Co. (Ann Arbor, MI). Troglitazone and farnesol were kindly provided by Drs. S. Jackson and P. Edwards (University of California, Los Angeles), and 1,25-dihydroxyvitamin D3 by Dr. D. Bikle (University of California, San Francisco). All other compounds were from Sigma Chemical Co. (St. Louis, MO). Fatty acids were added to medium bound to 0.5% (wt/vol) BSA. Isoprenoids and 25-hydroxycholesterol were added in ethanol, and clofibric acid (δ-chlorophenylisobutyric acid) and JH III were added in DMSO. Control explants were incubated in the presence of the appropriate vehicle (± 0.1% DMSO or ethanol, and/or ± 0.5% BSA).

Light and electron microscopy. Samples for light microscopy were fixed in modified Karnovsky’s solution, plastic-embedded, and 0.5-μm sections were stained with Toluidine blue. Samples for electron microscopy were fixed in 1-mm³ pieces, fixed in modified Karnovsky’s fixative, and postfixed in ruthenium tetroxide as described (7). Ultrathin sections were stained with uranyl acetate and lead citrate, and examined using an electron microscope (10A; Carl Zeiss, Inc., Thornwood, NY) operated at 60 kV.

Tissue preparation for enzyme assays. Epidermis was separated from dermis after incubation in 10 mM EDTA in Ca²⁺- and Mg²⁺-free PBS, pH 7.4, at 37°C for 30–40 min. The tissues were then minced, and homogenized on ice (3 × 15 s with a Polytron homogenizer, followed by 2 × 10 s sonication at 35% power) either in PBS containing 0.1 mM PMSF and 0.1% Triton X-100 (for β-glucocerebrosidase) or in 10 mM Tris (pH 7.5) containing 0.15 M sucrose and 2 mM EDTA (for steroid sulfatase). β-Glucocerebrosidase activity was measured in the supernatant after centrifugation at 10,000 g for 15 min at 4°C. Steroid sulfatase activity was measured in the microsomal fraction resulting from a 10,000 g centrifugation (10 min, 4°C) followed by 60 min 100,000 g ultracentrifugation (4°C).

β-Glucocerebrosidase and steroid sulfatase assays. β-Glucocerebrosidase activity was assayed using the synthetic substrate 4-MUG as described previously (11). Briefly, assays were performed in 5 mM sodium taurocholate in citrate-phosphate buffer (pH 5.6) with 0.5 mM 4-MUG for 60 min at 37°C, with a final assay volume of 100 μl, and protein concentration of 1–2 mg/ml. The reaction was terminated with 2 ml of carbonate-bicarbonate buffer (pH 10.5). The fluorescence was then measured at 360 λ (excitation) and 450 λ (emission) and compared with a standard (4MU) curve.

Steroid sulfatase activity was measured as described previously (12). Briefly, 100 μg of microsomal protein in 0.1 M Tris containing 5.6 mM glucose (pH 7.4) was incubated with 15 μM [3H]dihydroepiandrosterone sulfate (15 μCi) in a final volume of 1.1 ml. The product, [3H]dihydroepiandrosterone, was extracted with benzene and an aliquot counted by scintillation spectrophotometry.

Statistical analysis. Statistical evaluation was performed using a Student’s t test.

Results

Ligands/activators of RXR heterodimers which do not accelerate barrier development. We first sought to determine whether ligands or activators of members of the nuclear receptor superfamily, which dimerize with the RXR, alter the kinetics of barrier development. Skin explants from gestational day 17 rats were incubated in the presence of either various activators or vehicle, and transepidermal water loss was measured after 2 d.

Figure 1. Effect of activators of the RXR superfamily on barrier development in vitro. Full-thickness skin explants from gestational day 17 rats were incubated for 2 d in the presence of either 1 μM all-trans RA, 1 μM 9-cis RA, 1 μM 1,25 dihydroxy vitamin D3, 10 μM prostaglandin J3, 30 μM linoleic acid, or in control media, and transepidermal water loss was measured at the termination of the experiment, as described in Methods. Results are presented as mean±SEM of two separate experiments (n = 8). *P < 0.005.
Earlier studies have shown that full-thickness skin from gestational day 17 rats, after 2 d in culture, exhibits lamellar bodies in the granular cells, and secreted lamellar material in the SC interstices, but, like day 19 rat skin in utero, the SC lacks mature lamellar membrane structures and a competent barrier is not present (8). In contrast, an SC with barrier function equivalent to that observed in mature epidermis normally forms by day 4 in culture, corresponding to day 21 in utero (8). As shown in Fig. 1, 9-cis retinoic acid (RA) (RXR ligand), all-trans RA (RAR or RXR ligand), 1,25-dihydroxyvitamin D₃ (ligand for vitamin D receptor), or prostaglandin J₂ (PPARγ ligand) had no significant effect on barrier development. Furthermore, no effect on the rate of barrier development was observed with either 9-cis or all-trans RA, or 1,25 dihydroxyvitamin D₃, in concentrations ranging from 1 nM to 1 μM (data not shown). These data suggest that activators of the retinoid receptors, vitamin D receptor, and PPARγ alone do not accelerate fetal barrier development.

Activators of PPARα accelerate barrier development. Whereas retinoids and vitamin D₃ did not accelerate barrier development, activators of the PPARα markedly stimulated barrier formation. For example, water loss rates declined markedly in explants incubated in 30 μM linoleic acid for 2 d (Fig. 1; P < 0.005, n = 8). The three PPAR subtypes, α, δ, and γ, are pharmacologically distinct, and are differentially activated (19–21, 26, 27). Thus, while linoleic acid is a potent activator of the PPARα, it can also activate the δ isoform (21). To determine whether the acceleration of barrier development by linoleic acid is likely to be PPARα-mediated, we also tested a variety of other PPARα activators (Fig. 2). While several of these agents (oleic acid, WY 14,643, and ETYA), like linoleic acid, may also activate the δ isoform, clofibrate activates only the α isoform (21). As shown in Fig. 2, activators of the PPARα, such as the peroxisome proliferators, clofibrate, WY 14,643, and oleic acid, all significantly accelerated epidermal barrier development. Although toxic effects of the PPARα activator arachidonic acid precluded study, the synthetic arachidonic acid analog, ETYA (5,8,11,14-eicosatetraynoic acid), also a known PPARα activator (19, 21), induced significant acceleration of barrier development. In contrast, the PPARγ activator troglitazone, like prostaglandin J₂, did not accelerate barrier formation. Taken together, these data suggest that activators of PPARα, but not PPARγ, accelerate epidermal barrier development.

Activators of FXR accelerate barrier development. We next assessed whether activators of FXR would also accelerate barrier development. As seen in Fig. 1, all-trans farnesol significantly accelerated barrier ontogenesis (P < 0.005, n = 8). To determine whether the effect on barrier development by farnesol, an isoprenoid product of the mevalonate pathway (28), might be FXR-mediated, we also tested the effects of a variety of cholesterol analogs and precursors, as well as other isoprenoids, including the known FXR activator, JH III (23), on the kinetics of barrier development. As shown in Fig. 3, JH III, an FXR activator not present in mammalian cells, significantly accelerated barrier formation (P < 0.005, n = 6). In contrast, neither mevalonate, 25-OH cholesterol, nor squalene accelerated barrier formation (Fig. 3). Similarly, incubation of explants with the isoprenoids, geranylgeraniol, cis-farnesol, or nerolidol, did not significantly affect barrier function (Fig. 3). These results suggest that the effect of farnesol on barrier formation is unlikely to be related to its role as a precursor to cholesterol or other isoprenoid products, and that the acceleration of barrier development by farnesol and JH III is FXR mediated.

The effects of farnesol and clofibrate are additive. We next examined dose responses for the PPARα activators clofibrate, oleic acid, and linoleic acid, and for the FXR activators farnesol and JH III. As shown in Fig. 4 A, the acceleration of barrier formation by clofibrate and oleic acid is dose dependent, with maximal effects seen with 300 μM and half-maximal effects with ~150 μM. Linoleic acid (Fig. 4 A, inset) also accelerates

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**Figure 2.** Effect of PPARα activators on barrier development in vitro. Explants were incubated for 2 d in the presence of 100 μM 5,8,11-eicosatetraynoic acid (ETYA), 300 μM clofibrate, 100 μM WY 14,643, 300 μM oleic acid, 10 μM troglitazone, or in control media, and water was loss measured as in Fig. 1. Results are presented as mean±SEM of two separate experiments (n = 8). *P < 0.005.

**Figure 3.** Effect of FXR activators and isoprenoid precursors and metabolites on barrier development in vitro. Explants were incubated for 2 d in the presence of 100 μM nerolidol, geranylgeraniol, cis-farnesol, 25-hydroxy cholesterol, or squalene, or 200 μM mevalonate, or in control media, and water loss was measured as in Fig. 1. Results are presented as mean±SEM of two separate experiments (n = 8). *P < 0.005.
barrier formation in a dose-dependent manner, but it was >10-fold more potent than clofibrate or oleic acid, with maximal and half-maximal effects seen with 20 and 15 μM, respectively. Fig. 4B shows the dose responses for farnesol and JH III. In these experiments, farnesol was more potent than JH III (farnesol: 50 and 20 μM, JH III: 250 and 100 μM, maximal and half-maximal effective concentrations, respectively). The micromolar concentrations of the fatty acids and farnesol used here are consistent with their estimated plasma or intracellular concentrations and with levels known to activate PPARα or FXR (19, 21, 23), suggesting that these lipids may be endogenous activators of these receptors.

We next sought to determine whether 9-cis RA, the ligand for the RXR, together with PPARα or FXR activators might exert synergistic or additive effects on barrier development. No effect on either barrier function or epidermal morphologic maturation was observed with 9-cis RA (1 μM) in combination with either clofibrate (100 μM) or farnesol (10 μM) (data not shown), doses of the PPARα and FXR activators which alone did not accelerate barrier formation (see Fig. 4, A and B). In contrast, when clofibrate (100 μM) and farnesol (10 μM) were provided together in suboptimal concentrations, barrier development was significantly accelerated (Fig. 5A). However, incubation of explants with both activators at their maximally effective concentrations (farnesol and clofibrate, 50 and 300 μM, respectively) did not further accelerate barrier development over either agent given alone (Fig. 5B). The additive effects of clofibrate and farnesol at suboptimal doses suggest that both classes of activators may utilize a similar activation pathway. Furthermore, the lack of synergy between 9-cis RA and either clofibrate or farnesol suggests either that RXR activation is already present.

**PPARα or FXR activators stimulate epidermal structural maturation.** To examine the effects of PPARα and FXR activators on epidermal maturation, we next evaluated the morphology of explants incubated in control media or in media containing PPARα or FXR activators. As shown in Fig. 6A, the epidermis in control explants after 2 d in culture lacked a multilayered stratum granulosum or a distinct SC, corresponding to day 19 of gestation (7, 8). Similar to the functional results noted above, neither 1,25 dihydroxyvitamin D₃, all-trans RA, nor 9-cis RA altered structural maturation (compare with Fig. 1). In contrast, both a multilayered stratum granulosum and SC were observed in explants treated with either clofibrate (300 μM) (Fig. 6B) or JH III (100 μM) (Fig. 6C). Similar results were observed in explants treated with oleic acid (300 μM), linoleic acid (30 μM), and farnesol (50 μM) (not shown). In contrast, explants incubated with either clofibrate or farnesol in suboptimal concentrations did not exhibit a distinct SC and were morphologically indistinguishable from controls (data not shown).

We next compared the ultrastructural maturation of the outer epidermis using ruthenium tetroxide postfixation to delineate SC membranes in explants exposed to PPARα or FXR activators (Fig. 7). Multiple arrays of mature lamellar membrane unit structures fill the extracellular domains of the SC in clofibrate-treated (Fig. 7A, arrows) or JH III–treated (Fig. 7B, arrows) explants. Similar alignment of SC membrane deposition was observed in oleic acid–, linoleic acid–, or farnesol-treated explants (not shown). In contrast, the extracellular lamellae in the single layered SC in control explants were not yet organized into mature lamellar membranes (Fig. 7C). Thus, stimulation of the functional development of the barrier is accompanied by accelerated epidermal stratification and the more rapid appearance of mature lamellar unit structures in the SC intercellular regions.

Clofibrate and JH III induce β-glucocerebrosidase and steroid sulfatase activities, enzymatic markers of barrier maturation. Earlier studies have demonstrated that epidermal β-glucocerebrosidase and steroid sulfatase activities, enzymatic markers of barrier maturation.
Effect of PPARα and FXR activators alone and in combination on barrier development in vitro. Fetal skin explants were incubated for 2 d in the presence of suboptimal concentrations (A) or optimal concentrations (B) of clofibrate and farnesol, alone or in combination. Transepidermal water loss was measured at the termination of the experiment as described in Methods. Data represent the mean values of two experiments, n = 8. *P < 0.01 (clofibrate plus farnesol in comparison to control, and to clofibrate alone and to farnesol alone [A]).

cocerebrosidase activity increases during the final stages of barrier development in the rat in utero and in vitro, and that inhibition of this enzyme both prevents barrier formation and disrupts barrier homeostasis (11, 29). Moreover, hormones which accelerate epidermal barrier formation increase β-glucocerebrosidase activity in fetal skin explants (11). Likewise, steroid sulfatase activity also increases during the final stages of barrier formation and its activity also is stimulated by these hormones (12). To determine whether the activity of these two enzymes is affected by PPARα or FXR activators, we incubated 17-d fetal rat skin explants for 2 d in the presence of clofibrate (300 μM) or JH III (100 μM) and measured enzyme activity. As shown in Table I, β-glucocerebrosidase activity was approximately twofold higher in treated explants than controls after 1 (P < 0.005, n = 5) or 2 d (P < 0.005, n = 5). Moreover, steroid sulfatase was also increased significantly (1.6-fold over controls after 1 d of incubation, P < 0.01, n = 5, and 2.5-fold after 2 d, P < 0.005, n = 5). These data demonstrate that both clofibrate and JH III accelerate the developmental increases in activity of two lipid metabolic enzymes associated with barrier formation.

Discussion

The epidermal permeability barrier forms late in gestation. In earlier studies, we demonstrated that several hormones which act via nuclear receptors, notably glucocorticoids, thyroid hormone, and estrogen, accelerate barrier maturation (8–10). Yet, the role of hormonal ligands in normal barrier ontogenesis is not entirely clear, because fetal rat skin cultivated in vitro in hormone-free media develops a competent barrier following the same time course that is observed in utero (8). This observation prompted us to examine whether endogenous lipid activators of other nuclear hormone receptors might regulate barrier ontogenesis.

In this study, we have shown that activators of the PPARα (oleic acid, linoleic acid, or clofibrate) and of the FXR (farnesol or JH III) accelerate barrier development, as evidenced by decreased transepidermal water loss, increased epidermal stratification, and the appearance of mature lamellae in the extracellular spaces of a multilayered SC by day 2 of culture. Additionally, PPARα or FXR activators stimulate the activities of both β-glucocerebrosidase and steroid sulfatase, two key enzymes of mature SC function which are induced during the final stages of barrier ontogenesis (11, 12). Thus, functional, morphologic, and enzymatic markers of barrier development are accelerated by PPARα or FXR activators. Moreover, several of these activators, e.g., oleic and linoleic acid and farnesol, are candidates as physiologic activators of these receptors. We have shown previously that adult epidermis is an extremely active site of cholesterol and fatty acid synthesis (25), rivaling the liver, gut, kidney, and steroidogenic tissues. Moreover, lipid synthesis is high in fetal rat epidermis immediately

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Table I. Effects of PPARα or FXR Activators on β-Glucocerebrosidase or Steroid Sulfatase Activity in Fetal Skin

<table>
<thead>
<tr>
<th>Activity</th>
<th>n mol/μmol/min</th>
<th>μmol/min/mg</th>
<th>pmol/h/mg</th>
</tr>
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<tbody>
<tr>
<td>24-h incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.35±0.22</td>
<td>5.99±0.80</td>
<td></td>
</tr>
<tr>
<td>Clofibrate</td>
<td>2.97±0.30</td>
<td>13.90±1.10</td>
<td></td>
</tr>
<tr>
<td>JH III</td>
<td>2.89±0.20</td>
<td>12.55±1.25</td>
<td></td>
</tr>
<tr>
<td>48-h incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.02±0.45</td>
<td>8.75±0.91</td>
<td></td>
</tr>
<tr>
<td>Clofibrate</td>
<td>5.35±0.89</td>
<td>22.51±2.60</td>
<td></td>
</tr>
<tr>
<td>JH III</td>
<td>5.20±0.62</td>
<td>18.23±2.35</td>
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</table>

Explants were incubated for 24 or 48 h in the presence of 300 μM clofibrate, 100 μM JH III, or vehicle alone. Enzyme activity was measured as described in Methods. Results are presented as mean±SEM and represent two separate experiments. *P ≤ 0.005 compared with controls of same time point, n = 5.
before the formation of a measurable barrier to water loss (24). Thus, significant levels of fatty acids, i.e., activators of PPARα, and isoprenoids, i.e., cholesterol biosynthetic metabolites which activate the FXR, are likely to be generated in fetal epidermis at the critical day 17-19 stage in barrier maturation.

Farnesol is an isoprenoid metabolite of farnesyl pyrophosphate, which is involved in protein isoprenylation and in the regulation of the rate-limiting enzyme of the cholesterogenic pathway, HMG CoA reductase (30, 31). Although farnesol inhibits phosphatidylcholine synthesis in human leukemia cells, leading to apoptosis (32, 33), and inhibits vasoconstriction in arteries (34), no effects of farnesol or its metabolites on skin have been described before our studies. Despite both the identification of FXR in several extracutaneous tissues and the

![Figure 6](image6.png)

**Figure 6.** Effect of PPARα or FXR activators on the morphology of developing fetal skin in vitro. Explants were epon-embedded and 0.5-μm sections were stained with Toluidine blue. Explants incubated for 2 d in control media (Con, A) exhibit a multilayered stratum spinosum, but the stratum granulosum and SC are not yet well-defined. 2 d of incubation in 300 μM clofibrate (Clo, B) results in the appearance of a multilayered stratum granulosum and SC (brackets). Similar results are seen in explants incubated for 2 d in 100 μM JHIII (JH, C). Results shown are representative of three separate experiments. Bar, 8 μm.

![Figure 7](image7.png)

**Figure 7.** Effect of PPARα and FXR activators on ultrastructural maturation of fetal epidermis in vitro. Ruthenium tetroxide postfixation reveals mature lamellar unit structures (closed arrows) in the extracellular spaces of the SC in JH III–treated (A) or clofibrate-treated (B) fetal skin explants. Mature lamellae are not present in the extracellular spaces of the SC in control explants (C). Although secreted lamellar material (open arrows) is found in the SC interstices of control explants, it is not reorganized into mature structures. Bar, 0.25 μm.
identification of certain of its activators, the actual ligands, as well as the function of this signaling pathway, remain unknown. We provide evidence here that the effects of farnesol on epidermal permeability barrier development may be FXR-mediated, because: (a) farnesol is a known activator of FXR (23); (b) JH III, a sesquiterpenoid which regulates insect development in concert with ecdysteroids, and which also activates FXR (23), also significantly accelerated epidermal barrier development in these studies; and finally, (c) related iso-prenoids, such as nerolidol and cis-farnesol, and cholesterol precursors, such as mevalonate, or metabolites, such as 25-hydroxycholesterol, all of which do not activate the FXR, displayed no effects on barrier development. Thus, it is likely that the effects of farnesol and JH III on epidermal development are FXR-mediated rather than posttranscriptional, metabolic effects. The precise role of the FXR in barrier development awaits functional studies in which the FXR is either deleted or altered.

Other members of the RXR superfamily of nuclear hormone receptors appear to be important in fetal skin development. It has long been known that thyroid hormone stimulates markers of postnatal epidermal development in rodents such as incisor eruption and eye opening (35). Moreover, our laboratory has shown that thyroid hormone also accelerates fetal epidermal barrier ontogenesis (8). Furthermore, vitamin A and its analogues exhibit profound effects on fetal epidermal development (36, 37). Finally, recent studies have shown that overexpression of a mutated RARα in fetal murine skin results in aberrant epidermal development (17, 18).

In light of the above, it is somewhat surprising that neither all-trans RA, which activates the RAR, nor 9-cis RA, the natural ligand for RXR, had any effect on barrier development. Likewise, 1,25-dihydroxyvitamin D₃, which exerts changes in epidermal differentiation and proliferation (38, 39), had no effect on barrier development. This lack of effects does not exclude a role for one or more of these compounds in epidermal development. Although explants are maintained in a serum- and hormone-free medium, sufficient levels of endogenous vitamins A and/or D may be present in day 17 fetal skin to permit vitamin-dependent development to proceed. In addition, the principal effects of one or more of these vitamins could occur at an earlier or later time in gestation. Additionally, it is possible that this in vitro explant model does not precisely mirror all aspects of in utero ontogenesis. Although treatment with RA plus PPAR or FXR activators has been shown to synergistically induce gene expression in some experimental systems (23), we did not observe such synergism with the combination of retinoids and PPARα or FXR activators. We did observe, however, additive effects by activators of PPARα and FXR. Thus, clofibrate and farnesol, when added in concentrations which alone did not affect barrier development, together accelerated barrier development. Whether this reflects a common mechanism of action or a convergence of signaling pathways remains to be determined.

Although the genes regulated by FXR are yet to be identified, many of the genes regulated by PPARs are known to encode enzymes of lipid metabolism (20). Thus, PPARα and FXR activators may regulate the generation of the lipid constituents of the barrier during fetal development. In these studies, we have shown that PPARα and FXR activators induce expression of two lipid metabolic enzymes, β-glucocerebrosidase and steroid sulfatase, which are linked to barrier development and to SC homeostasis (11, 12, 30). Whether these enzymes contain regulatory sequences for these transcription factors and are induced directly, or whether the activators stimulate the expression of these enzymes indirectly remains to be determined. Finally, the role of these nuclear receptor activators in the regulation of barrier homeostasis in the adult also remains to be determined.

It is noteworthy that PPARα activators may also regulate surfactant formation in the lung, a tissue with many similarities to the skin. Both lung and skin provide a lipid-enriched interface between an aqueous and gaseous environment; both mature during precisely the same late gestational period; and both are developmentally accelerated by glucocorticoids, thyroid hormone, or estrogen (40–42). Pertinently, both organs rely on a lamellar organelle for lipid storage and secretion, contained within the granular cells in epidermis and in the alveolar type II cells in the lung. In the adult rat, clofibrate treatment results in increased quantities of lamellar bodies in alveolar type II cells (43). Interestingly, glucocorticoids, which accelerate both lung and epidermal development, strongly induce PPARα gene expression in liver at the transcriptional level (44, 45). Taken together, these data indicate a putative role for PPARα in lung and skin development. Thus while ongoing clinical trials have focused on whether maternal thyroid hormone and glucocorticoid treatment accelerates lung maturation in the very premature human infant, our current studies in vitro suggest that a number of other factors, such as PPARα and FXR activators, may also play important roles in accelerating epidermal and/or lung development. Because clofibrate has been shown to improve psoriatic lesions in adults (46), it is possible that topical application of PPARα and FXR activators to the skin of premature infants may also be effective.

In summary, we have shown that activators of the nuclear receptors PPARα and FXR accelerate epidermal barrier development, suggesting a physiologic role for these receptors in the regulation of cutaneous development. Further understanding of these signaling pathways and their interactions will undoubtedly provide valuable insights into the multifactorial regulation of epidermal development and may lead to new therapies which accelerate epidermal barrier maturation.

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