Pathogenesis of the cutaneous phenotype in inherited disorders of cholesterol metabolism
Therapeutic implications for topical treatment of these disorders

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Key words: barrier function, cholesterol, ichthyosis, statin, CHH syndrome, CHILD syndrome, desmosterolosis, SC4MOL, SC5D, SLOS

Molecular geneticists tend to conceptualize disease pathogenesis from the mutated gene outward, an approach that does not take into account the impact of barrier requirements in determining disease phenotype. An ‘outside-to-inside’ perspective has provided quite different explanations for the ichthyoses, including several of the disorders of distal cholesterol metabolism. Elucidation of responsible pathogenic mechanisms also is pointing to appropriate, pathogenesis (pathway)-based therapeutic strategies. In the case of the lipid metabolic disorders, it takes full advantage of new molecular, genetic and cellular pathogenesis information to correct or bypass the metabolic abnormality. This approach fully exploits the unique accessibility of the skin to a topical approach. Moreover, since it will utilize topical lipids and lipid-soluble, and often generic, lipid-soluble drugs, these treatments should be readily transported across the stratum corneum. If successful, this approach could initiate an entirely new departure for the therapy of the ichthyoses.

Introduction

Current treatment of the ichthyoses remains symptomatic, and largely directed towards reducing the scaling component of these disorders. Yet, such therapy of the ichthyoses is often irrational, because removal of excess scale can interfere with homeostatic responses that allow patients to survive in a harsh, terrestrial environment. Moreover, the favored alternative, corrective gene therapy, though seductive in concept, remains a distant dream, impeded by: (1) difficulties in transcutaneous drug delivery; (2) enormous costs of the required ‘designer gene’ approach; (3) discomfort of intracutaneous injections; and (4) unknown, long-term risks of transfection with viral vectors.

All ichthyoses, including inherited syndromic disorders of distal cholesterol metabolism, display a permeability barrier abnormality, with the severity of the clinical phenotype paralleling the prominence of the barrier abnormality (reviewed in ref. 1–3). In our research, we have assumed that the cutaneous phenotype represents a best attempt by a metabolically-compromised epidermis to generate a competent permeability barrier in the desiccating, terrestrial environment (op. cit.). While “normal” epidermis mounts a vigorous, metabolic response in response to a compromised barrier that rapidly restores function,4–6 “ichthyotic” epidermis only partially succeeds in normalizing function.1–3 As a result, the clinical phenotype reflects the negative consequences of the genetic mutation for epidermal function, coupled with the epidermis’ impaired, homeostatic response. Thus, unraveling the cellular and biochemical mechanisms that account for the barrier abnormality provides an explanation for the pathogenesis of the cutaneous phenotypes (reviewed in ref. 2, 3 and 7), and it could point to potentially-novel, pathway-based therapies. In disorders due to impaired cholesterol synthesis, evidence to date suggests that the clinical phenotype in most cases reflects either accumulation of toxic metabolites and/or deficiency of pathway end-product.8 Moreover, in all of the lipid-metabolic disorders, whether due to metabolite accumulation, pathway product...
deficiency, or both, lamellar/non-lamellar (L/NL) phase separation within the lamellar bilayers accounts, at least in part, for the barrier abnormality (examples of pathogenic mechanisms for disorders of distal cholesterol metabolism are shown in Table 1).

We typically assess four different, functional end-points; i.e., TEWL, pH, hydration and stratum corneum (SC) integrity, which separately or together can impact permeability barrier function. For example, a less cohesive SC, which often is due to an elevated pH of SC, increases proteolytic degradation of corneodesmosomes, which results in a poor quality SC. A high pH also activates serine proteases, which degrade lipid processing enzymes. Finally, the pH-driven increase also activates pro-inflammatory cytokines, such as IL-1α and IL-1β, further aggravating barrier function while provoking inflammation.

Together, this approach has identified key pathophysiologic abnormalities (e.g., metabolite accumulation and/or product depletion) in these disorders, which in turn could point to the most-promising, potential, pathogenic-based therapeutic interventions (see below).

**Pathogenesis of Multisystem, Cholesterol Biosynthetic Disorders**

Nine enzymatic steps are required to generate cholesterol from lanosterol, with the further generation of cholesterol sulfate from cholesterol comprising a tenth step (Fig. 1). While syndromic disorders, with a variety of developmental malformations, have been reported in seven of these diseases,6-13 an abnormal cutaneous phenotype has been described in only six, i.e., lathosterolosis, desmosterolosis, Congenital Hemidysplasia with Ichthyosiform features.16

The pathogenesis of the ichthyosiform dermatosis (and likely the extracutaneous abnormalities) in all of the inborn errors of distal cholesterol metabolism can be variously attributed to either: deficiency of cholesterol in cell membranes and/or toxic effects of accumulated sterol precursors with resulting functional alterations.18,19 Sterol precursors can only partially substitute for cholesterol in the formation of SC lamellar membranes, and cholesterol is one of the three key SC lipids (along with ceramides and free fatty acids) required to form lamellar membranes (reviewed in ref. 20). Additional, downstream pathogenic mechanisms whereby sterol metabolites could contribute to disease pathogenesis include: (1) formation of oxysterol metabolites that either downregulate cholesterol synthesis or activate the liver X receptor;21 (2) altered hedgehog pathway signaling (HOX normally is tethered onto cell membranes via a cholesterol moiety);22-25 and/or (3) deficient peroxisomal function, as we have described in both CHH, CHILD patients and in the ‘bare patches’ mouse model24-28 which displays Nidhi mutations that mimic CHILD syndrome29,30 (further information about our work on the pathogenesis of CHILD syndrome, CHH and XLI is provided below); and (4) sterol metabolite-accelerated degradation of HMGCoxA reductase.31

| Disorders | Keratohyalin/ Keratins | Lamellar body formation/ Contents | Lamellar body exocytosis | Post-secretarylipid processing | Lamellar bilayers | Cornified envelopes | Corneodesmosomes | Cornocyte lipid envelope |
|-----------|------------------------|---------------------------------|-------------------------|-------------------------------|------------------|-------------------|------------------|----------------------|
| CHH/CHILD9,29 | Normal/Normal | Abnormal contents | Impaired | Delayed | L/Non-L-P5 | Normal | Normal | Normal |
| XLI37 | Normal/Normal | Normal | Normal | Normal | Normal | Normal | Persist | Normal |

*Bolded and italicized features are particularly helpful in differential diagnosis.*
Availability of mouse models of CHH and CHILD syndrome should allow for an assessment of the mechanistic basis for the cutaneous phenotype in CHH and CHILD syndrome, and for preclinical evaluation of potential therapies for these patients.

Both the density of lamellar bodies (LB) and LB secretion appear normal in CHH, but organelle contents are abnormal, displaying vesicular inclusions. Moreover, newly-secreted material fails to disburse at the stratum granulosum-SC interface. Importantly, maturation of lamellar bilayers is delayed, and bilayer membranes with normal morphology are displaced by extensive areas of lamellar/non-lamellar phase separation (Table 1). Yet, the morphology of clinically-affected skin sites in CHILD syndrome (OMIM #308050), however, differs in its distribution from CHH; i.e., it is strictly unilateral, including both skeletal defects and internal organ involvement. Skin lesions are circumscribed plaques, surmounted by prominent wax-like scales, which typically involve flexures.

CHH is caused by mutations in EBP (emopamil-binding protein) that encodes 3β-hydroxysterol-Δ8, Δ7-isomerase, which catalyzes the conversion of 8(9)-cholestenol to lathosterol, resulting in diagnostic elevations in serum 8-dehydrocholesterol and 8(9) cholesterol. Mutations in NSDHL, which encodes a member of the enzyme complex that removes the C-4 methyl group from lanosterol, underlie CHILD syndrome. However, CHILD syndrome can also be caused by mutations in EBP (Fig. 1). Given the close proximity of the sites of metabolic blockade, the presence of some phenotypic overlap between CHILD and CHH is not surprising (reviewed in ref. 9). Availability of mouse models of CHH and CHILD syndrome should allow for an assessment of the mechanistic basis for the cutaneous phenotype in CHH and CHILD syndrome, and for preclinical evaluation of potential therapies for these patients.

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which then are largely (but incompletely) secreted.7 Nevertheless, the SC displays a huge expansion of the extracellular matrix, which is filled with interspersed lamellar and non-lamellar material.7 Together, these features predict a severe barrier abnormality in both CHILD and CHH.

Recessive X-linked ichthyosis (XLI). The pathogenesis of XLI is better known than for any of the other ichthyoses. As a result of steroid sulfatase (Ssase) deficiency in XLI, cholesterol sulfate (CSO4) accumulates in the outer epidermis, 44-46 in erythrocyte cell membranes, 45,47 as well as in both the LDL (β-lipoprotein) and pre-LDL fractions of plasma.1 But CSO4 levels in epidermis are an order of magnitude higher than are levels in blood, 45,47 likely explaining the prominence of epidermal vs. other organ involvement in XLI.48 Normally, CSO4 levels decline to about 1% of lipid mass in the outer SC, through ongoing hydrolysis during SC transit.49,50 In contrast, the SC in XLI typically contains 10–12% cholesterol sulfate (by dry weight).48 Hydrolysis of CSO4 generates some of the cholesterol required for the barrier, while conversely, CSO4 itself is a potent inhibitor of HMGCoA reductase, further reducing cholesterol levels in XLI.48 Although Ssase is secreted from lamellar bodies (like other lipid hydrolases that process barrier lipid precursors into their own hydrophobic products), CSO4 is delivered to the SC interstices by its extreme amphiphilicity, which allows it to diffuse readily across cell membranes;51 i.e., in the absence of a lipid milieu within corneocytes, cholesterol sulfate likely partitions preferentially into the highly hydrophobic, extracellular domains of the stratum corneum. Accumulation of CSO4 coupled with cholesterol depletions, provokes lamellar/non-lamellar phase separation,17 accounting for the barrier abnormality in XLI.52

Recent Studies in Relevant Animal Models

Insig-2 (Epi-insig) DKO mice. The Brown and Goldstein group recently published relevant work on another animal model with aberrant cholesterol synthesis; i.e., epidermal-localized Insig-1 mice with an additional germ-line deletion of Insig-2 (Epi-Insig) DKO mice.53 This model mimics the syndromic human disorder, Ichthyosis Follicularis, Atartricia and Photophobia (IFAP) syndrome (OMIM #308205). Deletion of these intracellular proteins allows migration of SREBP from the endoplasmic reticulum to the Golgi apparatus followed by translocation to the nucleus, where these SREBPs excessively stimulate several genes involved in cholesterol synthesis. As a result, both sterol metabolites and cholesterol accumulate in the skin. Pertinent to these mice, response to treatment with topical simvastatin, which simultaneously normalizes both metabolite production and cholesterol levels in epidermis.53 This recent work, coupled with preliminary evidence of efficacy with topical lovastatin plus cholesterol in infantile lathosterola, 54 provides evidence that metabolite accumulation in the epidermis can be toxic; and conversely, that blockade of metabolite production (and normalization of cholesterol levels) could be beneficial for these disorders.

Mouse models of SLOS. While SLOS patients display a minimal skin phenotype, Dher7-/- mice display prominent ichthyosis, with neonatal lethality due to a putative permeability barrier abnormality.56 More pertinent to SLOS patients, who display residual enzyme function, our still-unpublished preliminary studies in Dher7-/- mice (with S. Patel) demonstrate epidermal structural abnormalities in both lamellar body contents and lamellar bilayer organization, predictive of a barrier abnormality in SLOS patients with comparable reductions in enzyme activity. These mice also display serum 7DHC and cholesterol levels that are comparable to patients with moderate SLOS due to partial loss-of-function mutations.56 One relatively-common mutation in SLOS (T93M) has been recapitulated in a transgenic ‘knock-in’ mouse model.57,58 While their skin phenotype has not yet been assessed, this model should also be useful to assess both pathogenic mechanisms and preclinical therapeutic studies.

Mouse models of CHH, CHILD, desmosterolosis and lathosterolosis. We also have recently begun to assess a potential animal model of desmosterolosis. Like Dher7-/- mice, Dher24-/- mice are neonatal lethal, apparently due to a failure of epidermal development in utero, but possibly also due to a barrier defect.56,57 In contrast, Dher24-/- mice survive, and like Dher7-/- mice, they show structural evidence of a skin barrier abnormality. While the Bpa, Stri and Tattered strains closely mimic varying severities of CHH and CHILD syndromes, one of these animal models is not a pure analogue of human disease; i.e., the Sc5d-/- mouse, but partial loss-of-function in the Sc5d-/- could reflect comparable reductions in enzyme function in lathosterolosis. Thus, this model could also be useful to assess pathogenic mechanisms and potential therapies for lathosterolosis.

Therapeutic Implications

Current therapy of the ichthyoses is largely aimed at scale removal; i.e., it is purely symptomatic. Not only is this form of therapy relatively ineffective, it often can be counterproductive; e.g., by removing ‘excess’ stratum corneum (SC), symptomatic therapy can do more harm than good (=therapeutic paradox’) as seen with: (1) retinoids in the treatment of Netherton syndrome (NS),58 and sometimes in epidermolytic ichthyosis (EI);59 (2) excessive absorption of salicylates, lactic acid or immunosuppressive molecules in CHH and CHILD syndromes, one of these animal models is not a pure analogue of human disease; i.e., the Sc5d-/- mouse, but partial loss-of-function in the Sc5d-/- could reflect comparable reductions in enzyme function in lathosterolosis. Thus, this model could also be useful to assess pathogenic mechanisms and potential therapies for lathosterolosis.
it would seem reasonable to deploy a proximal enzyme inhibitor [e.g., of HMGC\(\text{CoA}\) reductase (lovastatin)] to reduce levels of potentially-toxic metabolites. In addition, provision of the pathway product (cholesterol) to avoid epidermal dysfunction due to cholesterol deficiency could also be beneficial by further downregulating (negative feed-back) of HMGC\(\text{CoA}\) reductase activity. Then, this new information about disease pathogenesis has the potential to be deployed rapidly into topical therapy for patients with rare inherited disorders of distal cholesterol metabolism (Table 2). Another not-so-rare, inherited disorder of distal cholesterol metabolism, X-linked ichthyosis (XLI), occurs in 1:2,000–6,000 males. Our studies over several years already have shown that disease pathogenesis in XLI also reflects both metabolite (CSO4) accumulation and end-product (cholesterol) deficiency (see also above).17,46,48 Pertinently, topical cholesterol alone in our experience is not effective in XLI, but the ichthyosis in XLI could be treatable with either a topical statin or a sulfotransferase inhibitor (plus cholesterol). It must be emphasized that blockade of metabolite production alone, though it could be temporarily useful, cannot be utilized as monotherapy for the cutaneous phenotype in these disorders, because the end-product of this pathway (i.e., cholesterol) is required to prevent development of a permeability barrier abnormality (topical statins provoke a barrier abnormality with ichthyosiform changes in normal mouse skin).61,62

In very preliminary studies, we have treated two patients with one of the rare disorders of distal cholesterol metabolism (CHILD syndrome) [Drs. Amy Paller (Northwestern University) and Marina Rodriquez-Martin (Canary Islands Univ Hospital)—point mutation in G83Dp Gly83 Asp in \(\text{NSHDL}\), and nonsense mutation (c.317C>A; p.S106x) in \(\text{NSHDL}\), respectively]. Notably, these patients failed to improve with topical cholesterol alone, but both responded to dual treatment with cholesterol plus lovastatin.54 Both excessive scale and epidermal hyperplasia diminished greatly, and one patient displayed improved mobility of underlying extremities by 6–8 weeks of treatment. In principal, this approach bridges the two poles of symptomatic vs. curative (i.e., gene) therapy. It also displays the following inherent advantages: (1) it is disease-targeted and mechanism-based specificity; (2) it is inherent safe; (3) it is relatively low-cost; and (4) most-importantly, it exploits the accessibility of skin to assess efficacy, as well as providing the opportunity to assess the mechanisms responsible for positive outcomes.

Yet, topical pathogenesis-based therapy is not curative, and even if successful, it would need to be utilized for the duration of the patient’s lifetime. Moreover, despite knowledge of disease pathogenesis, it is possible that such mechanism-targeted
therapies may not always be effective, if multiple downstream pathways contribute to disease pathogenesis. However, since these patients’ skin displays higher than normal water permeability, both the inhibitors and lipid end-products should be readily bioavailable. If either the drug or the lipid end-product fails to be delivered (suggested by a failure to respond to either pathway product or pathway product + inhibitor), it is possible that applications of inhibitor and lipid product together could interfere with absorption of one or both.

Finally and importantly, it is possible that there will be benefits for the extracutaneous features of one or more of these disorders. In the case of disorders of distal cholesterol metabolism, the transdermal approach has the potential to deliver statins and cholesterol to extracutaneous tissues prior to hepatic first-pass metabolism, offering the tantalizing prospect that topical therapy could improve the extracutaneous manifestations in one or more of these disorders. It is highly likely that the cutaneous phenotype reflects pathogenic mechanisms that also are on-going in extracutaneous tissues, successful pathogenesis-based therapy for the ichthyoses could point to comparable approach(es) to treat/prevent the extracutaneous manifestations of these disorders.

Acknowledgements

We are indebted to Joan Wakefield for superb editorial and graphic assistance. These studies were supported by NIH grant AR019098 and the Medical Research Service, Department of Veterans Affairs, San Francisco, CA.

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