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Role of cholesterol sulfate in epidermal structure and function: Lessons from X-linked ichthyosis[☆]

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Abstract

X-linked ichthyosis is a relatively common syndromic form of ichthyosis most often due to deletions in the gene encoding the microsomal enzyme, steroid sulfatase, located on the short arm of the X chromosome. Syndromic features are mild or unapparent unless contiguous genes are affected. In normal epidermis, cholesterol sulfate is generated by cholesterol sulfotransferase (SULT2B1b), but desulfated in the outer epidermis, together forming a 'cholesterol sulfate cycle' that potentially regulates epidermal differentiation, barrier function and desquamation. In XLI, cholesterol sulfate levels may exceed 10% of total lipid mass ($\approx 1\%$ of total weight). Multiple cellular and biochemical processes contribute to the pathogenesis of the barrier abnormality and scaling phenotype in XLI. This article is part of a Special Issue entitled The Important Role of Lipids in the Epidermis and their Role in the Formation and Maintenance of the Cutaneous Barrier. Guest Editors: Kenneth R. Feingold and Peter Elias.

Keywords

Epidermal barrier function; Epidermal lipid metabolism; Cholesterol sulfate; Corneodesmosomes; Steroid sulfatase; X-linked ichthyosis

1. Introduction

X-linked ichthyosis (XLI) (OMIM #308100) is due to loss-of-function mutations in the gene that encodes the microsomal enzyme, steroid sulfatase (SSase; STS) [1–5]. Female carriers rarely exhibit a skin phenotype [6,7], probably because the region of the X chromosome where STS resides escapes X-inactivation. Affected males present at birth, or shortly thereafter, with generalized peeling or exaggerated neonatal desquamation, although some may exhibit a collodion membrane [8,9].

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2. Clinical features

After the neonatal period, fine scaling persists on the trunk and extremities, but over time, scales often become coarser and darker. While scaling is generalized, it typically spares the antecubital and popliteal fossae, palms, soles, and the mid-face, but the lateral face, axillae and the neck always remain involved.

The clinical features of XLI bear some similarities to *ichthyosis vulgaris* (IV), a common, autosomal semi-dominant trait caused by mutations in the filaggrin gene. However, the darker color of the scale and its more ‘centripetal’ distribution, as well as the sparing of the palms and soles, point to a clinical diagnosis of XLI [9]. Yet, in the absence of an X-linked pedigree, phenotypic overlap with other mild-to-moderate ichthyosis requires further studies to definitively establish the diagnosis of XLI. Moreover, because IV and the xerosis associated with atopic dermatitis (AD) are both quite common, the two disorders may co-exist, producing a more severe phenotype in affected patients [10–12]. Indeed, both of these disorders are relatively-common (XLI occurs in 1:1,800; filaggrin mutations occur in up to 10% of the European population). In a recent series of 11 Korean XLI patients, 7 had a prior history of atopic disease, while only one displayed flexural involvement, a reliable clinical marker of AD. Thus, *filaggrin* represents a genetic modifier of the XLI phenotype.

Routine histopathology in XLI typically shows moderate hyperkeratosis with mild acanthosis and partial accentuation of the granular cell layer. While these features are nonspecific, they can help to exclude IV or filaggrin-deficient AD, which should instead display decreased keratohyalin granules. Measurement of substrate accumulation in skin (cholesterol sulfate) or blood (cholesterol sulfate or other sulfated steroid hormones) is diagnostic, as is the assay of SSase activity in epidermis [13,14], cultured fibroblasts, or leukocytes [15,16]. Serum lipoprotein electrophoresis is also diagnostic, demonstrating more rapid mobility of the LDL (beta) and pre-LDL (pre-beta) fractions due to an increase in sulfated sterol content [15,17]; however, this assay is no longer widely available. Because most XLI cases arise from deletion of the STS gene [18–26], fluorescence *in situ* hybridization (FISH) analysis is commonly employed for diagnosis of XLI and its carrier state [27], but FISH testing provides false negatives in XLI patients who have point mutations ($\approx 10\%$ of affected XLI subjects).

3. Syndromic features of XLI

XLI is considered a systemic, albeit usually mild, syndromic disorder [28]. *Placental sulfatase deficiency syndrome* (PSD), which occurs in pregnancies of XLI fetuses, can manifest as failure of labor either to initiate or to progress, defective cervical softening, and a poor response to exogenous pitocin. PSD syndrome can be detected prior to the development of these complications by low maternal urinary and blood estriol levels due to the placenta’s (a largely fetal structure) failure to desulfate estrone sulfate [19,29,30]. Since maternal estriol levels are part of the so-called ‘triple screen’ employed to detect pregnancies at risk for trisomies 18/21, Smith–Lemli–Opitz syndrome, and neural tube defects, many XLI fetuses are now detected in this manner [31–34].

In contrast to earlier estimates, the incidence of *cryptorchidism* (testicular maldescent) does not appear to exceed 5–10% [35,36]. Moreover, earlier reports of *testicular cancer* in a few XLI patients with normally descended testes [37] have not been confirmed as a true association. These associations instead could be due to a contiguous gene syndrome, with loss of pseudouridine-phosphate phosphatase [38]. Small, entirely asymptomatic, comma-shaped *corneal opacities* develop in the posterior capsule of Descemet’s membrane in $\approx 25\%$ of adult XLI patients, but these often are not present in affected children. But when

present, these opacities are diagnostic, and some female carriers display similar opacities [9,36]. A small number of XLI patients have developed *nephrotic syndrome*, attributable to the deleterious effects of excessive cholesterol sulfate on glomerular cell function [38]. Finally, in recent reports, as many as 40% of XLI patients demonstrated cognitive behavioral abnormalities, such as *attention-deficit disorder* [39,40], but the actual incidence may be closer to 25%. These abnormalities have been attributed to altered sterol metabolism in the central nervous system [40–42]. Inactivation of SSase increases aggressive behavior in rodents [43], providing some further support for this new association.

Because the STS locus lies on the distal tip of the short arm of the X chromosome, deletion of this locus is often accompanied by additional deletions of flanking regions, resulting in a series of contiguous gene syndromes. In these instances, XLI males present with an ichthyosiform phenotype in the setting of multisystem disease [44–47]. One commonly reported association is with Kallman's syndrome, displaying anosmia, hypogonadism. *Mental retardation*, linked to deletions in the neighboring VCX gene has also been observed [48–50], as have contiguous gene syndromes with chondrodysplasia punctata, autism cerebellar ataxia, and albinism. In one large series, 8% of STS-deficient fetuses exhibited deletions of additional contiguous genes [51].

4. Molecular Biology and Regulation of SSase

SSase (EC 3.1.6.2, arylsulfatase-C) is a member of a superfamily of 12 different mammalian sulfatases that hydrolyze alkyl steroid sulfates (e.g., dehydroepiandrosterone sulfate [DHEAs]) and aryl steroid sulfates (e.g., estrone sulfate) to their unconjugated forms (Fig. 1). The STS gene that encodes SSase, which is located distally on the short arm of the X chromosome, consists of 10 exons that span 146 kb, while the cDNA encodes a protein with 583 amino acids, as well as four potential glycosylation sites [52]. The promoter region of the SSase gene is unusual in that it resembles neither a housekeeping gene nor other tightly-regulated genes, and also lacks binding sites for Sp1 and other common transcription factors [53]. It is located proximal to the portion of the gene that encodes enzyme expression, but the promoter sequence varies in a tissue-specific fashion [54]. While cytokines such as TNF α and IL-6 upregulate enzyme activity, IL-1 β instead reportedly downregulates SSase activity [55], although IL-1 β (as well as interferon γ) reportedly down-regulate SSase expression by inhibiting NF κ B, while activating the glucocorticoid receptor [55]. Finally, both retinoids and 1,25(OH) $_2$ vitamin D3 induce both SSase activity and expression [56].

5. Enzyme characteristics and epidermal localization

SSase is a 65 kDa microsomal enzyme that localizes to the endoplasmic reticulum, Golgi, and endosomal membranes, including coated pits (but not in lysosomes) of placenta and several other tissues [52,57,58]. A key feature of SSase is that exogenous substrates, such as estrone sulfate [59] and cholesterol sulfate [60], induce enzyme activity. In addition, specific, high-affinity sterol sulfate transporters can be activated by their substrates [61].

In normal epidermis, SSase protein and enzyme activity are low in the basal and lower spinous layers, but both increase in the outer nucleated cell layers, where they peak in the granular layer [14]. Enzyme activity persists into the stratum corneum, where it continues to desulfate cholesterol sulfate, contributing to the pool of cholesterol available to form the extracellular lamellar bilayers (Figs. 2 & 3). In ultra-structural cytochemical studies, SSase activity localizes not only within the cytosol, but also within lamellar bodies, followed by its exocytosis from lamellar bodies into the interstices of the lower stratum corneum [62] (Fig. 3). Thus, SSase, like other lipid hydrolases that are involved in the processing of polar lipids to more hydrophobic species in the stratum corneum, utilizes the lamellar body secretory

system to reach the extracellular domain, where it can participate in the regulation of permeability barrier homeostasis and desquamation [63]. In contrast to SSase, cholesterol sulfate exploits its extreme amphiphilicity to diffuse into the extracellular domains from the cytosol of granular cells ([64]; see also below). Finally, while we are focusing here on cholesterol sulfate, epidermal SSase also could increase the bioavailability of androgens not only in epidermis, but also in hair follicles [65,66], where it has been implicated in the pathogenesis of androgenetic alopecia [67].

6. Cholesterol sulfotransferase

Cytosolic sulfotransferases (SULTs) represent a superfamily of enzymes that catalyze the sulfoconjugation of hormones, neurotransmitters, drugs, xenobiotics, and sterols [68,69]. The SULT superfamily of enzymes is composed of five families, of which the SULT2 family is primarily responsible for the sulfation of endogenous steroids and sterols. The SULT2 family is further divided into SULT2A1 and SULT2B1. SULT2A1 catalyzes the conversion of DHEA to DHEA sulfate and is commonly referred to as DHEA sulfotransferase. The SULT2B1 subfamily consists of two isoforms, SULT2B1a and SULT2B1b, derived from the same gene via differential splicing. The SULT2B1a isoform preferentially sulfonates pregnenolone, but not cholesterol, while SULT2B1b preferentially catalyzes the conversion of cholesterol to cholesterol sulfate. Thus, the SULT2B1b isoform accounts for the majority of cholesterol sulfotransferase activity. The human SULT2B1b gene is localized to chromosome 19, where it encodes a protein that contains 365 amino acids [69].

In the epidermis and in keratinocytes SULT2B1b is expressed, while neither SULT2B1a nor SULT2A1 is observed by either PCR or Western blotting [70]. The expression of SULT2B1b and cholesterol sulfotransferase activity increases in keratinocytes subjected to calcium-induced differentiation [70]. Using immunocytochemistry, SULT2B1b is observed in both the basal and suprabasal layers of the epidermis [70,71]. Retinoic acid, which inhibits differentiation, also inhibits cholesterol sulfotransferase activity, while PPAR α , PPAR β /d, PPAR γ , and LXR activators, which stimulate differentiation, instead increase SULT2B1b expression and cholesterol sulfotransferase activity [72–74]. Similarly, TPA and TNF, which also stimulate keratinocyte differentiation, increase cholesterol sulfotransferase activity [74–76]. Finally, both EGF and IGF-1 have been shown to increase cholesterol sulfotransferase activity in keratinocytes [75].

Cholesterol sulfotransferase (SULT2B1b) activity generates cholesterol sulfate in the lower nucleated cell layers of the epidermis, while in contrast SSase peaks in the outer epidermis (stratum corneum) (Fig. 2). Hence, Epstein et al. [77] proposed that an ‘epidermal cholesterol sulfate cycle’ exists in the epidermis in which cholesterol is first sulfated in the lower epidermis, and then desulfated back to cholesterol in the outer epidermis. Thus, the epidermal content of cholesterol sulfate increases from 1% to 5% of total lipid as nucleated epidermal cells move from the basal to the granular layer and then declines again to 1% as corneocytes move, from inner to outer stratum corneum (SC) [78,79] (Fig. 2). Disruption of this cholesterol sulfate cycle accounts for both the abnormal desquamation, as well as the permeability barrier abnormality in XLI (see below).

7. ‘Cholesterol sulfate cycle’ and its regulatory significance

Cholesterol sulfotransferase (SULT2B1b) activity generates cholesterol sulfate predominately in the lower nucleated cell layers of the epidermis, while in contrast SSase peaks in the outer epidermis (Fig. 2). Epstein et al. (1984) proposed an ‘epidermal cholesterol sulfate cycle’ in which cholesterol is first sulfated in the lower epidermis, and then desulfated back to cholesterol in the outer epidermal nucleated layers. Thus, cholesterol

sulfate increases from 1% to 5% of the total lipid content as nucleated epidermal cells move from the basal to the granular layer and then declines again to 1% as corneocytes move, from inner to outer stratum corneum (SC) [78,79] (Fig. 2). Disruption of this cholesterol sulfate cycle accounts for both the abnormal desquamation, as well as the permeability barrier abnormality, in XLI (see below).

Sulfation of cholesterol by cholesterol sulfotransferase (SULT2B1b) is intimately linked to epidermal differentiation [79,80,81] and formation of the stratum corneum [70,72,82]. Not only does the content of cholesterol sulfate and the activity of cholesterol sulfotransferase increase with keratinocyte differentiation (*vide supra*), levels of cholesterol sulfate are much higher in keratinizing vs. mucosal epithelia [81]. Conversely, reversal of keratinization (through induction of mucous metaplasia in keratinizing epithelia, following application of exogenous retinoids) dramatically reduces tissue cholesterol sulfate levels [72,83]. Moreover, both SULT2B1b expression and cholesterol sulfate levels increase late in epidermal development *in utero* [84,85], in parallel with the formation of a functionally-competent stratum corneum [86].

The increase in cholesterol sulfate that occurs in conjunction with keratinocyte differentiation may not be just a marker of differentiation but may in fact be a signaling molecule that plays a role in inducing keratinocyte differentiation. Adding cholesterol sulfate to keratinocytes in culture or overexpressing SULT2B1b, which increases cholesterol sulfate formation, stimulates keratinocyte differentiation [87,88]. Cholesterol sulfate is a potent transcriptional regulator in both cutaneous and extracutaneous tissues [68,69], stimulating epidermal differentiation by several mechanisms (Fig. 4): 1) It activates the η isoform of protein kinase C (PKC) [89–91], which in turn stimulates the phosphorylation of epidermal structural proteins, while also increasing cornified envelope formation. 2) it is a transcriptional regulator of several proteins involved in cornified envelope formation, such as transglutaminase 1 (TGM1) and involucrin, operating through an AP-1 binding site in the promoter region of these proteins [92,93]. Hanley et al demonstrated that cholesterol sulfate increased the expression of Fra-1, Fra-2, and Jun D, members of the AP-1 family of transcription factors. It is likely that these two mechanisms are linked, because PKC activation by cholesterol sulfate could phosphorylate AP-1 transcription factors, leading to enhanced transcriptional regulation of differentiation-linked proteins, such as TG-1 and involucrin [94] (Fig. 4). 3) recent studies have shown that cholesterol sulfate induces the expression of filaggrin through the induction of ROR α [88]. Cholesterol sulfate both increases the expression of ROR α and serves as a ligand for ROR α increasing its transcriptional activity. Together, these observations provide biochemical and molecular mechanisms whereby cholesterol sulfate could impact epidermal differentiation.

While the above studies demonstrate that the addition of cholesterol sulfate stimulates keratinocyte differentiation recent studies have further shown a physiologic role for cholesterol sulfate formation in regulating keratinocyte differentiation. Shimada and colleagues used shRNA to down regulate the expression of SULT2B1b in keratinocytes in culture demonstrated that concomitant with a decrease in cholesterol sulfate levels there was a decrease in involucrin expression. Furthermore, using shRNA they were able to demonstrate that decreasing SULT2B1b expression in mouse epidermis also reduced involucrin expression [87]. As described above TPA treatment of keratinocytes or mouse skin stimulates both SULT2B1b and involucrin expression. If keratinocytes in culture or mouse skin are treated with shRNA to prevent the TPA induced increase in SULT2B1b the ability of TPA to increase involucrin expression is markedly blunted. Together these observations suggest that the formation of cholesterol sulfate in the epidermis may not only be a marker of differentiation but may also be an important signaling molecule.

8. Basis for the phenotype in XLI

Likely because cholesterol sulfate levels are an order of magnitude higher in epidermis than in blood [15,16], that the skin phenotype in XLI is more prominent than that in other organs [95]. Whereas in normal stratum corneum, cholesterol sulfate levels decline from $\approx 5\%$ to about $\approx 1\%$ of lipid mass in the outer SC [14,78,96] (Fig. 2), in XLI, cholesterol sulfate is 10–12% of the lipids in the stratum corneum [14,95]. Because lipids as a group account for $\approx 10\%$ of the dry weight of stratum corneum, $\approx 1\%$ of tissue mass in XLI is cholesterol sulfate!

While most extracellular lipids and enzymes in the SC are delivered via the secretion of lamellar body contents, cholesterol sulfate is not concentrated in lamellar bodies [62,97]. Its mode of delivery to the stratum corneum interstices is uncertain, but it is likely that this highly amphiphilic molecule can move across cell membranes by simple diffusion, preferentially partitioning into the lipid-enriched extracellular domains [64].

Alternatively, cholesterol sulfate could be actively delivered from the stratum granulosum cells into the extracellular space by specific transporters that are known to transport sulfated conjugates of lipophilic compounds (ABCC1, ABCC3, and/or ABCC4 [MRP1, MRP3, MRP4]) [98]. These transporters are expressed in keratinocytes and the level of expression has been shown to increase with differentiation [99].

Lamellar body density and content appear normal in XLI, as are the corneodesmosomes in the lower stratum corneum. The key ultrastructural features that explain the ichthyosiform phenotype in XLI include: i) the persistence of “pristine” corneodesmosomes, with little evidence of degradation in the outer layer of the stratum corneum (Figs. 4 & 5), leading to an abnormally cohesive SC and secretion, while ii) disruption of the lamellar bilayers opens a pathway for the outward diffusion of water. The presence of frequent, focal sites of electron-dense, non-lamellar material disorganizes the extracellular lamellae [62,100] (Fig. 6). A defective permeability barrier would in turn stimulate epidermal hyperplasia, resulting in the formation of additional layers of corneocytes. These two processes together likely explain the hyperkeratotic phenotype in XLI.

9. Basis for the permeability barrier abnormality in XLI

While patients with XLI display only a mild barrier abnormality under basal conditions [101–103], the kinetics of barrier recovery slow significantly following acute perturbations [100], suggesting that the excess cholesterol sulfate in the stratum corneum in XLI destabilizes permeability barrier homeostasis. In support of this hypothesis, excess cholesterol sulfate forms non-lamellar domains in both model lipid mixtures [104,105], and in XLI scale [106] (Fig. 6). Yet, the barrier abnormality in XLI could also be due in part to the decreased cholesterol content of the stratum corneum in XLI (reduced by approximately 50%) [95] (Fig. 5). In experimental animals, a comparable decrease in cholesterol results in formation of abnormal extracellular lamellar membranes, producing a barrier abnormality [107]. This decrease in cholesterol content of XLI SC may be due to a reduced generation of cholesterol from cholesterol sulfate [100,108], and/or to cholesterol sulfate-mediated inhibition of HMGCoA reductase, the rate-limiting enzyme of cholesterol synthesis [108] (Fig. 5). In summary, the dominant mechanisms that account for the barrier abnormality in XLI appear to be: 1) lamellar/non-lamellar phase separation due to excess cholesterol sulfate; and 2) reduced cholesterol content of the stratum corneum lamellar membranes [62]. While more severe barrier defects in other ichthyoses result in marked epidermal hyperplasia and inflammation, the lesser abnormality in XLI provokes little epidermal hyperplasia or inflammation. This minimal pathology is supported by molecular assay studies that show very few genes are altered in the epidermis of XLI patients [103].

10. Cellular mechanisms account for abnormal desquamation in XLI

Kinetic studies have demonstrated that the hyperkeratosis in XLI largely reflects delayed desquamation [109]. The basis for this classic, retention-type of ichthyosis is persistence of corneodesmosomes at all levels of the SC (Fig. 5). Two key serine proteases, kallikrein 7 (Klk 7, SC chymotryptic enzyme [SCCE]) and kallikrein 5 (Klk 5, SC tryptic enzyme [SCTE]) mediate the initial stages of corneodesmosome degradation [110]. Cholesterol sulfate increases SC retention through its known function as a serine protease inhibitor [62,111,112] (Fig. 5). Moreover, while the acidic pH of stratum corneum inhibits Klk 5 and 7 activities [113–115], the pH of the stratum corneum in XLI is even more acidic than normal [116], further reducing Klk activity in the SC of patients with XLI [62].

The stratum corneum in XLI demonstrates abundant Ca^{++} in extracellular domains, which preferentially localizes along the external faces of opposing corneodesmosomes [62] (Fig. 6). Thus, the delayed degradation of corneodesmosomes in XLI could be due in part to leakage of Ca^{++} into the lower SC (due to the barrier defect), with formation of Ca^{++} bridges between adjacent corneodesmosomes [62]. If Ca^{++} is present in sufficient quantities, it could stabilize the highly-anionic sulfate groups (from persistent cholesterol sulfate) in the extracellular lipids [77]. Indeed, cholesterol sulfate-containing liposomes aggregate avidly in the presence of Ca^{++} [117,118]. Thus, multiple mechanisms likely contribute to the abnormal desquamation in XLI (Fig. 5).

11. Conclusions

X-linked ichthyosis is an inherited syndromic disorder in which an ichthyosiform phenotype predominates. A broad array of extracutaneous tissue may be affected, but these features are often unapparent or extremely mild, except when continuous genes are affected. The cutaneous clinical phenotype can be explained by the impact of excess cholesterol sulfate on epidermal differentiation and lipid synthesis, as well as on the organization of the lamellar lipids that provide the permeability barrier, and the ability of this lipid to inhibit corneodesmosomes proteolysis. One of the paradoxes about XLI that is still not fully understood is the multiplicity of biological roles and pathological effects of cholesterol sulfate vs. the relative mildness of the clinical phenotype.

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Abbreviations

CSO₄	cholesterol sulfate
FISH	fluorescence <i>in situ</i> hybridization
IV	ichthyosis vulgaris
Klk	kallikreins
PSD	placental sulfatase syndrome
SC	stratum corneum
SCCE	stratum corneum chymotryptic enzyme
SCTE	stratum corneum tryptic enzyme

SSase	STS, steroid sulfatase
SULT2B1b	cholesterol sulfotransferase
XLI	x-linked ichthyosis

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Fig. 1.
Steroid sulfatase desulfates cholesterol sulfate and other sulfated steroid hormones (modified from Elias, et al., 2010.).

Fig. 2.
Epidermal Functions Impacted by Cholesterol Sulfate Cycle (modified from Elias, et al., 2004, 2010).

Fig. 3. Steroid sulfatase (SSase) activity in lamellar bodies (B) in stratum granulosum (SG) and within the extracellular spaces of the stratum corneum (SC) (A, arrows). Method for cytochemical detection of SSase activity can be found in Ref. #62, Elias, et al., 2004).

Fig. 4.
Potential Pathogenetic Mechanisms In X-linked Ichthyosis (modified from Elias, et al., 2004, 2010).

Fig. 5.
Pathogenesis of X-Linked Ichthyosis (modified from Elias, et al., 2010).

Fig. 6. Extracellular Matrix Abnormalities in XLI. A: Ruthenium tetroxide postfixation, which reveals lamellar/non-lamellar (electron-dense) phase separation; and B: Ca^{++} leakage into the SC interstices (consistent with barrier abnormality) where it localizes to the external faces of corneodesmosomes (pyroantimonate precipitation method for Ca^{++} detection). Mag bars = $0.2 \mu\text{m}$ (A) and $0.1 \mu\text{m}$ (B). (Modified from Elias, et al., 2010.)