Topical Liver X Receptor Activators Accelerate Postnatal Acidification of Stratum Corneum and Improve Function in the Neonate

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In neonatal rat stratum corneum (SC), pH declines from pH 6.8 at birth to adult levels (pH 5.0–5.5) over 5–6 d. Liver X receptor (LXR) activators stimulate keratinocyte differentiation, improve permeability barrier homeostasis, and accelerate the *in utero* development of the SC. In this manuscript we determined the effect of LXR activators on SC acidification in the neonatal period and whether these activators correct the functional abnormalities in permeability barrier homeostasis and SC integrity/cohesion. Formation of the acid SC-buffer system was accelerated by topically applying the LXR activator, 22(*R*)-hydroxycholesterol, and non-oxysterol activators of LXR, TO-901317, and GW-3965. A sterol which does not activate LXR had no effect. LXR activation increased secretory phospholipase A_2 ($sPLA_2$) activity and conversely, inhibition of $sPLA_2$ activity prevented the LXR induced increase in SC acidification, suggesting that increasing $sPLA_2$ accounts in part, for the LXR stimulation of acidification. LXR activation resulted in an improvement in permeability barrier homeostasis, associated with an increased maturation of lamellar membranes attributable to an increased β-glucocerebrosidase activity. SC integrity cohesion also normalized in LXR-activator-treated animals and was associated with an increase in corneodesmosomes and in desmoglein 1 expression. These results demonstrate that LXR activators stimulate the formation of an acidic SC and improve both permeability barrier homeostasis and SC integrity/cohesion.

Key words: acidification/barrier function/barrier homeostasis/cohesion/desmoglein 1/integrity/liver X receptor activators (LXR) /neonatal Rat/pH/stratum corneum
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That the surface of the skin is acidic (acidic stratum corneum (SC)-buffer system) has been recognized for decades (Heuss, 1892; Schade, 1928; Marchionini and Hausknecht, 1938; Blank, 1939; Bernstein and Hermann, 1942; Draize, 1942; Arbenz, 1952; Behrendt and Green, 1958; Beare *et al*, 1960; Jolly, 1960; Baden and Pathak, 1967; Tippelt, 1969; Braun-Falco and Korting, 1986; Zlotogorski, 1987; Korting *et al*, 1990; Seidenari and Giusti, 1995; Berardesca *et al*, 1998; Yosipovitch *et al*, 1998; Eberlein-Konig *et al*, 2000; Fluhr *et al*, 2000), but the mechanisms that account for its acidification are still not completely understood. It is postulated that exogenous pathways (originating outside the epidermis), such as microbial metabolites (Di Marzio *et al*, 1999), free fatty acids of pilosebaceous origin (Puhvel *et al*, 1975; Bibel *et al*, 1989), and eccrine gland-derived products (Ament *et al*, 1997), such as lactic acid (Thueson *et al*, 1998), contribute to SC acidification. Additionally, recent studies have shown that endogenous pathways are also important for SC acidification (Fluhr *et al*, 2001a, 2004a; Behne *et al*, 2002). Generation of *cis*-urocanic acid from histidine (Schwarz *et al*, 1986; Krien and Kermici, 2000), free fatty acid generation from phospholipid hydrolysis catalyzed by secretory phospholipase A_2 ($sPLA_2$) (Mao-Qiang *et al*, 1996; Fluhr *et al*, 2001a, 2004a), and a sodium/proton pump antiporter, sodium/hydrogen antiporter-1 (NHE1) (Behne *et al*, 2002, 2003; Fluhr *et al*, 2004a), have all been shown to play a role in SC acidification.

Both a fully developed cornified envelope (Hardman *et al*, 1998; Komuves *et al*, 1998; Lee *et al*, 1999) and abundant extracellular lamellar membranes (Hanley *et al*, 1997; Williams *et al*, 1998; Hanley *et al*, 1999) are generated in the SC late in gestation. Hence prior to birth, the fetus develops a cutaneous permeability barrier sufficient for survival in a terrestrial environment. But skin surface pH is neutral at birth both in humans and in rodents (Behrendt and Green, 1958; Visscher *et al*, 2000; Yosipovitch *et al*, 2000; Fluhr *et al*, 2004a). In humans it takes several weeks to months before the SC is fully acidified whereas in the neonatal rat the SC attains normal adult pH levels over the first postnatal week (Behrendt and Green, 1958; Visscher *et al*, 2000; Yosipovitch *et al*, 2000; Fluhr *et al*, 2004a). Recent studies by our laboratory have shown in rodents that $sPLA_2$ activity

Abbreviations: 22(*R*)-Chol, 22(*R*)-hydroxycholesterol; aSM'ase, acid sphingmyelinase; BPB, bromphenacylbromide; DMSO, dimethyl sulfoxide; DSG1, desmoglein 1; β-Gluc Cer'ase, β-glucocerebrosidase; LXR, Liver X receptor activators; NHE-1, sodium/hydrogen antiporter-1; SC, Stratum corneum; SG, stratum granulosum; $sPLA_2$, secretory phospholipase A_2; TEWL, transepidermal water loss

is low at birth but increases progressively after birth (Fluhr *et al*, 2004a). Moreover, inhibition of $sPLA_2$ activity delays postnatal SC acidification. The NHE1 antiporter is expressed at birth and inhibition of this transporter also delays SC acidification in the neonatal period (Behne *et al*, 2003; Fluhr *et al*, 2004a). Conversely, neither microbial colonization nor urocanic acid appears to play a key role in neonatal acidification (Fluhr *et al*, 2004a). Thus, the postnatal acidification of the SC can be attributed, at least in part, to $sPLA_2$ and NHE1 (Behne *et al*, 2003; Fluhr *et al*, 2004a) and $sPLA_2$ and NHE1 have been identified to be key sources of endogenous SC acidification.

Traditionally the major function of the acid SC-buffer system has been assumed to be antimicrobial (Aly *et al*, 1975; Hartmann, 1983; Bibel *et al*, 1989). But recent studies have demonstrated abnormalities in the function of the SC in the neonatal period, which are because of the lack of an acidic SC at birth (Fluhr *et al*, 2004a). Specifically, although basal permeability barrier function is competent in neonates, the recovery of permeability barrier function following acute barrier disruption is delayed (Fluhr *et al*, 2004b). The delay in barrier recovery was attributed to incompletely processed lamellar membranes in the SC because of a decrease in the activity of a key enzyme for lipid processing during SC maturation; β-glucocerebrosidase, an enzyme whose optimal activity is at an acidic pH. Both the abnormality in permeability barrier homeostasis and the structural abnormality of the lamellar membranes could be corrected by topical treatment with an acidic buffer, which normalized β-glucocerebrosidase activity (Fluhr *et al*, 2004b). In addition to the abnormality in permeability homeostasis, a decrease in SC integrity also was seen in newborn rats. The decrease in SC integrity was attributed to a decrease in the density of corneodesmosomes (CD), with an associated decrease in desmoglein 1 and corneodesmosin protein expression in the neonatal SC (Fluhr *et al*, 2004b). CD are key structures for SC cohesion and desmoglein 1 is one of the major proteins of CD. Alteration in CD density correlate with changes in SC integrity and cohesion. Topical treatment with an acidic buffer again increased the number of CD and restored SC integrity to normal (Fluhr *et al*, 2004b). Thus, the delay in acidification of neonatal skin results in functional abnormalities that could have adverse clinical consequences for the newborn.

Liver X receptor (LXR) are members of the nuclear hormone receptor superfamily, which form heterodimers with RXR, in order to activate gene transcription. Two genes, α and β, encode the LXR paralogues. LXRα is expressed predominately in the liver and to a lesser extent in the kidney, spleen, adrenal gland, and the small intestine (Willy *et al*, 1995), whereas LXRβ is ubiquitously expressed (Song *et al*, 1995). Our laboratory has shown that both LXRα and LXRβ are present in cultured human keratinocytes and in fetal rat epidermis (Hanley *et al*, 1999, 2000). LXRα and LXRβ are now recognized to bind oxysterols, including 22(*R*)-hydroxycholesterol (22(*R*)-Chol), 24(*S*)-hydroxycholesterol, and 24(*S*),25-epoxycholesterol. Activation of LXR regulates important pathways in cholesterol, fatty acid, and bile acid metabolism. Recently, we demonstrated that topical application of oxysterols to murine epidermis and the addition of oxysterols to human keratinocyte cultures stimulate keratinocyte differentiation (Fowler *et al*, 2003; Schmuth *et al*, 2004, 2005). In addition, topical oxysterol treatment of normal adult animals improves permeability barrier homeostasis following acute barrier disruption (Komuves *et al*, 2002). Furthermore, in an animal model of epidermal hyperplasia, oxysterol treatment restored epidermal morphology towards normal by both inhibiting proliferation and stimulating differentiation (Komuves *et al*, 2002). Finally, and of particular pertinence to this manuscript, oxysterols also accelerate the formation of the epidermal permeability barrier and stimulate differentiation during fetal development (Hanley *et al*, 1999, 2000). Thus, activation of LXR improves epidermal function in a variety of experimental models. We therefore hypothesized that treatment of neonatal rat skin with LXR activators could stimulate acidification and normalize the previously described abnormalities in cutaneous function that occur in the neonatal epidermis.

Results

Topical LXR activators accelerate postnatal acidification in neonatal rats In separate experiments, topical treatment of newborn mice for 3 d with three chemically unrelated LXR activators, an oxysterol, 22(*R*)-Chol, and two non-oxysterol pharmacologic activators of LXR, TO-901317 and GW 3965, stimulated the acidification of neonatal skin (Fig 1). In contrast, topical treatment with cholesterol, a

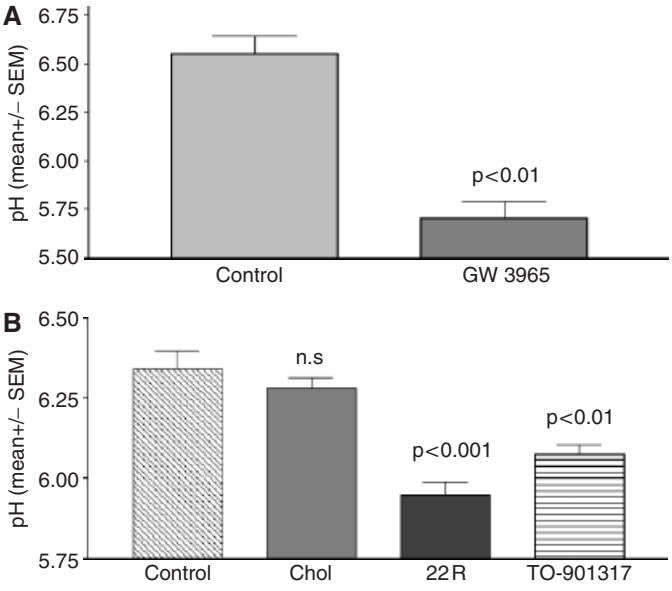

Figure 1
Topical treatment with liver X receptor (LXR) ligands accelerates acidification of the stratum corneum (SC). (*A*) Newborn animals were treated topically with GW 3965, an LXR ligand, twice a day for 3 d. On day 3, SC surface pH was measured (N = 7; mean ± SEM). (*B*) Newborn animals were treated topically with cholesterol, 22(*R*)-hydroxycholesterol or TO-901317 twice a day for 3 d. On day 3, SC surface pH was measured. For both sets of experiments the compounds were dissolved in acetone at a concentration of 10 mM. The newborn rats were treated on each flank twice per day over 3 d with 20 μL of the freshly prepared solutions. In order to prevent the mothers from licking the applied substances off the newborn rats, we placed the pups in a plastic container in a 37°C incubator for 2–3 h. The measurements were performed after 3 d of treatment (N = 7; mean ± SEM).