'BARRIER REPAIR' INGREDIENTS AND FORMULATIONS REGULATE BARRIER HOMEOSTASIS, AND ANTIMICROBIAL PEPTIDE EXPRESSION IN PARALLEL

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ABSTRACT

Prior studies have shown first, that permeability barrier homeostasis and cutaneous antimicrobial defense are co-regulated and interdependent functions. Second, we demonstrated that expression of the mouse analogue of the epidermal antimicrobial peptide, cathelicidin (LL-37), and to lesser extents, human β-defensin and catestatin, decline in parallel with compromised barrier function with skin aging, during psychological stress, and following erythemogenic doses of UV-B. Here, we explored the opposite scenario; i.e., whether ingredients or formulations that improve barrier homeostasis also enhance epidermal AMP expression, Accordingly, petrolatum (≥2%), urea (≥5%), PPAR and LXR activators, suberythemogenic UV-B, and a ceramide-dominant, triple-lipid mixture of cholesterol, free fatty acids, and ceramides (EpiCeram® emulsion) all accelerated barrier repair in normal human and/or mouse epidermis. In contrast, several other putative 'barrier repair' formulations either displayed no net benefits, or delayed barrier recovery after acute insults to human skin. For all of those ingredients and formulations that enhanced barrier repair, AMP expression was enhanced in parallel. These results show again the close relationship between permeability barrier function and epidermal antimicrobial defense. Agents that promote barrier function exert parallel benefits for cutaneous antimicrobial defense.

INTRODUCTION

Permeability and Antimicrobial Barriers are Linked, Co-Regulated, and Interdependent

- Co-localization in extracellular ('mortar') domains
- Pathogens attempt to invade through SC extracellular domains
- Some permeability barrier lipids (e.g., free fatty acids and sphingosine) exhibit potent antimicrobial activity
- At least two antimicrobial peptides (AMP) localize to lamellar bodies (along with lipids), and are co-delivered to SC extracellular domains
- AMP expression is upregulated and secretion of AMP accelerates after permeability barrier disruption, paralleling increases in lipid synthesis
- At least one AMP (LL-37) is required for permeability barrier homeostasis
- Cathelicidin expression is downregulated under conditions where barrier function is **impaired**, but much less information is available about changes in AMP expression when barrier function is **enhanced**.

Hypothesis: **Strategies that Enhance Barrier Function Should Increase Cathelicidin Production in Parallel.**

Approach: We evaluated changes in AMP expression following topical treatment of hairless mouse and/or human skin with various ingredients and formulations that enhance barrier function, including: 1) 5, 10, 20% urea; 2)two different LXR activators; 3) two different PPARα activators; 4) PPARβ/δ activator; 5) petrolatum; 6) triple-lipid, ceramide dominant physiological lipid mixture (EpiCeram® emulsion). EpiCeram was chosen, because it proved to be the sole, so-called 'barrier repair' formulation that accelerated barrier recovery in human skin (Fig. 1).

Table 1: CHANGES IN ANTIMICROBIAL PEPTIDE EXPRESSION IN RELATION TO ALTERED BARRIER FUNCTION

Permeability Barrier Status	AMP Expression		
Decreased	**mCAMP**	**mBD3**	**Cst**
Psychological Stress (PS)[1]	↓	↓	↑
Exogenous GC[1]	↓	↓	N/D
Testosterone-replete[2]	↓	No change	No change
Erythemogenic UV-B[2]	↓	(↓)	↑↑
Aging[2]	↓	↑	↑
Increased			
Sub-erythemogenic UV-B[3]	↑	↑	N/D
Urea[4]	↑	↑	N/D

Abbreviations: AMP, antimicrobial peptide; Cst, catestatin; GC, glucocorticoid; mBD3, mouse β-defensin 3; mCAMP, mouse cathelicidin antimicrobial peptide; N/D, not demonstrated
[1]Aberg, et al., 2007, [2]Rodriquez-Martin, et al., 2011, [3]Hong, et al., 2008, [4]Grether-Beck, et al., 2012.

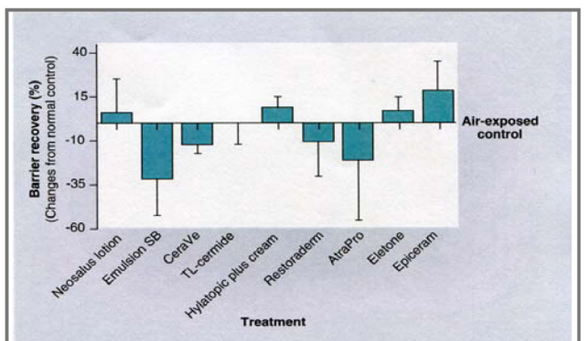

Figure 1: Nine (22–62-year olds; seven females and two males) non-atopic human volunteers were enrolled in this study. Creams (n = three to four subjects each) were applied to 3 × 3 cm areas (of previously untreated skin sites) on the flexural surface of the forearm twice daily for 4 days. Two sites, 12–14 cm apart, were selected on each forearm. Untreated sites on contralateral forearms served as normal controls. During the study period, no detergents or skin-care products were applied to the forearm flexors. On day 5, basal pH, hydration and transepidermal water loss were measured with a Tewameter®. Barrier recovery rates were assessed 3 h following barrier disruption by repeated tape stripping until transepidermal water loss levels ≥5 mg/cm²/h. Data are expressed as mean ± standard error of the mean. **(Elias, et al., Exp Rev Derm, 2013)**

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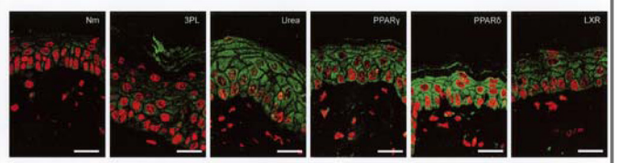

Fig. 2: Note upregulation of LL-37 immunostaining after 2 days of twice-daily applications of various unrelated barrier-enhancing preparations to normal hairless mouse skin. Mag bar = 10 μM.

Table 2: PARALLEL CHANGES IN BARRIER FUNCTION AND LL-37 EXPRESSION

Approaches	Basal Barrier Function	Barrier Recovery Kinetics	LL-37 (or mCAMP) expression
Sub-erythemogenic UV-B	Improves [1]	Accelerates [1]	↑
Cer-dominant triple lipid mixture	Improves [2]	Accelerates [2]	↑
Petrolatum	Improves[3]	Accelerates [3]	No change
PPARα	No change	Accelerates [4]	↑
LXR	No change	Accelerates [4]	↑
Urea (5, 10, 20%)	Improves [5]	N/D	↑

Abbreviations: Cer, ceramide; LXR, liver X receptor; mCAMP, mouse cathelicidin antimicrobial peptide; N/D, not demonstrated; PPAR, peroxisome proliferator-activated receptor.
[1]Hong, et al., 2008; [2]Man, et al., 2006, [3]Man et al., 1995, [4]Man, et al., 2004· [5]Grether-Beck, et al., 2012.

CONCLUSIONS

- **Several unrelated strategies that enhance barrier function increase cathelicidin (LL-37) expression in parallel.**
- **These results provide further support for the concept that permeability barrier function and epidermal antimicrobial defense are intertwined and co-regulated processes.**

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