Epidermal barrier improvement after Excipial application in atopic xerosis is revealed by non-invasive proteome analysis.

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INTRODUCTION

The *stratum corneum* plays a crucial role in barrier function and is under investigation in several skin pathologies. The *stratum corneum* (SC) of atopic xerosis (AX) reveals not only decreased hydration but also mildly impaired barrier function characterized by an increase in Trans Epidermal Water Loss (TEWL), elevated pH values, and an increased turnover rate of the SC consisting of thick layers of smaller-sized corneocytes (Tagami *et al*, 2006). The mildly impaired SC functions of AX can be improved by daily repeated applications of effective moisturizers, which are effective in preventing the progression of AX to atopic dermatitis (AD).

In a recent study performed on a cohort of 26 subjects (**Table 1**), clinical and biophysical observations were in favour of an improvement of skin surface after Excipial anti-itch foam application: TEWL, pH and other properties were normalised after 8 daily applications of Excipial anti-itch foam (**Table 2**). In addition, pruritus was also significantly decreased after 2 days (**Table 3**) (Lio, 2016).

OBJECTIVE

In AX the effect of moisturizers in general, and of Excipial in particular, on the SC protein composition has not yet been fully analysed. Using a simple and painless tape-stripping procedure, we investigated the proteome of SC to establish molecular effects of Excipial anti-itch foam in AX.

METHODS

Mass spectrometry analysis was performed using protein extracted from SC collected by tape stripping before and 48h after 8 days of Excipial anti-itch foam application. Western blot analysis and ELISA (Luminex) were performed to quantify serum albumin.

RESULTS

More than 400 proteins were identified in SC using label free mass spectrometry analysis (**Figure 1**). A change in the protein content before and after treatment was observed. Several proteins were found significantly modulated after Excipial treatment (**Table 4**). We found a significant increase in content of proteins involved in cohesion of the SC and its barrier function, such as Desmoplakin and the Keratin type II cytoskeletal 2 involved in the formation of the cornified envelope and proteins involved in regulation of epidermal homeostasis, such as filaggrin-2 (**Figure 2**). In addition, we found two abundant unique peptides of serum albumin significantly decreased after treatment (**Figure 3a**). Serum albumin was proposed as marker of extravasation which is increased in skin injuries and skin diseases. Western blot analysis revealed the presence of the intact form of albumin (**Figure 3b**). No notable degradation forms of albumin were observed in untreated or Excipial-treated skin. Using Luminex technology for the quantification of serum albumin, a slight decrease in serum albumin content was observed after Excipial treatment (fold: -1.5, p <0.05) (**Figure 3c**).

Other proteins described as markers of terminal differentiation, including proteases and endogenous protease inhibitors were also modulated after Excipial application (**Table 4 and Figure 4**). The recovery of the SC function using repetitive Excipial applications is supported by the increase of key proteins such as filaggrin 2 and Keratin, type II cytoskeletal 2 which were reported decreased in AD (Winget *et al*, 2016). Interestingly, albumin was previously described up-regulated in AD (Winget *et al*, 2016). Taken together, our proteomics data indicate that skin homeostasis and barrier function in AX was improved at the molecular level after 8 days Excipial anti-itch foam application.

(a) Atopic Xerosis (AX)
(Dry skin of subjects prone to atopic dermatitis)
26 subjects – Applications from Day 1 to Day 8 twice daily

(b)

Figure 1: proteomic workflow. (a) Sample collection and protein extractions: *Stratum corneum* was harvested through five repetitive tape-stripping on day 1 (without treatment) and Day 10 (48h after the last application) using D-Squames (Monaderm [Monaco] Ø 2.2 cm, ref: D100). Proteins were extracted from D-Squames in 600µl of phosphate buffered saline (PBS) containing 0.2% of Progenta™ Non-ionic Acid Labile Surfactant ALS-400 (*Proteabio*). **(b) bottom up Mass spectrometry analysis** (LC-MS/MS analysis). Mass spectrometry analysis was performed directly from crude protein extracts (50 µg of protein) using an Ultimate 3000 UH-PLC nanosystem (Dionex, Amsterdam, Netherlands) coupled to a Qexactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). *Mascot* and *Sequest* algorithms through *Proteome Discoverer* 1.4 Software (Thermo Fisher Scientific, Inc.) were used for protein identification in batch mode by searching against a Reference Proteome Set Homo sapiens database from the Uniprot website (release 2012_03). *Genedata* (Expressionist version 10.5) was used for LC-MS label-free intensity based quantitation as recommended by manufacturer using standard analysis as default. Dataset of the *Genedata* analysis using as parameters at least 2 peptides for quantification and a power > 0.8.

Figure 2: Desmoplakin, Keratin, type II cytoskeletal 2 and filaggrin 2 content in SC before (in blue) and after (in red) treatment with Excipial in the 24 subjects analysed. Relative abundances were calculated using Genedata software after normalization (geometric).

(a) Serum albumin peptides Fold change Anova P value

CCTESLVNR	-2.7	<0.01
QNCELFEQLGEYK	-6.8	<0.01

Figure 3: Serum albumin expression levels in stratum corneum before and after treatment. (a) Fold modulation of two major peptides of serum albumin quantified by LC-MS (label free) in *stratum corneum* at Day 1 (before treatment) versus Day 10 (after 8 days of everyday application). **(b)** Western blot analysis of serum albumin in *stratum corneum* from 5 to 40 µg of protein extract using the rabbit anti-albumin from Cell Signaling (4929). Western blot were performed with samples collected before and after Excipial treatment showing the same profile with an major band corresponding to expected apparent molecular weight of serum albumin: 68 kDa (arrow). (c) Quantification of serum albumin in *stratum corneum* using Luminex assay (L-6050, Millipore). Human serum albumin was found slightly decreased in treated dry atopic-like skin (Leg) after ten day with Excipial.

Table 4. Protein significantly modulated in stratum corneum after 8-day treatment with Excipial through mass spectrometry analysis.

Protein name	protein ID (Uniprot)	gene name	Total Peptides	Unique Peptides	Fold change [D10 vs D1]	Q-Value
Annexin A8	P13928	ANXA8	3	3	3.26	*
Desmoplakin	P15924	DSP	94	94	3.19	*
Filaggrin-2	Q5D862	FLG2	9	9	2.90	**
Glutathione S-transferase P	P09211	GSTP1	5	5	2.34	*
Keratin, type II cytoskeletal 2 epidermal	P35908	KRT2	36	24	2.26	*
Protein S100-A11	P31949	S100A11	4	4	2.22	*
Plakophilin-1	Q13835	PKP1	9	9	2.13	*
Fructose-bisphosphate aldolase A	P04075	ALDOA	9	8	1.74	*
Peroxiredoxin-6	P30041	PRDX6	3	3	1.65	*
Phosphoglycerate kinase 1	P00558	PGK1	10	10	1.49	*
Annexin A4	P09525	ANXA4	10	10	1.48	*
Vinculin	P18206	VCL	21	21	-1.50	*
Cystatin-A	P01040	CSTA	4	4	-1.70	*
Interleukin-36 gamma	Q9NZH8	IL36G	6	6	-1.73	*
Kallikrein-8	O60259	KLK8	4	4	-1.79	*
Proteasome subunit beta type-6	P28072	PSMB6	4	4	-1.91	**
AP-2 complex subunit beta	P63010	AP2B1	2	2	-2.13	*
DNA damage-binding protein 1	Q16531	DBP1	5	5	-2.22	*
Proteasome subunit alpha type-3	P25788	PSMA3	2	2	-3.21	*
Ig alpha-1 chain C region	P01876	IGHA1	7	3	-3.50	*
Superoxide dismutase [Cu-Zn]	P00441	SOD1	3	3	-4.92	**

* < 0.05; ** < 0.01

(a)

(b)

Protein name	modulation after Excipial application on XD skin	modulation in AD (Winget et al.)	function
Serum albumin	↓	↑	homeostasis, extravasation marker
Filaggrin-2	↑	↓	homeostasis, moisturizer factor
Keratin, type II cytoskeletal 2 epidermal	↑	↓	barrier function
Serpin B12 (ADLTGSPSPNYLSK)	↑	↓	homeostasis, protease inhibitor
Protein S100-A11	↑	↑	differentiation and cornification of keratinocytes
Desmoplakin	↑	↑	desmosomal protein

Figure 4 : (a) Epidermis homeostasis. At the upper layer of the *stratum corneum* (SC), each corneocyte must shed off. Degradation processes of cell-cell interactions (corneodesmosome) contribute to desquamation. Various proteases and glycosidase are secreted by differentiated keratinocytes in *stratum granulosum* in membrane-coating granule (MCG) and are involved in this process. The weakly acidic condition of the SC maintains the protease activity. Tryptic and chymotryptic serine proteases as Kalikreins 5 and 7 are involved in the degradation of extracellular part of desmoglein 1, desmocollin 1 and corneodesmosin. Filaggrin (as well as filaggrin-2) is also degraded in SC resulting in the production of free amino acids, which make up almost half of the natural moisturizing factors (NMFs) contributing to the epidermis hydration. Caspase-14, elastase, breomycin hydrolases are involved in filaggrin proteolysis. **(b) modulation in SC protein content in Atopic dermatitis** was reported previously. Interestingly, for several proteins the Excipial treatment after 10 days displays a normalised expression.

CONCLUSION

Excipial anti-itch foam efficacy on dry skin(AX) was revealed by the proteome analysis of *stratum corneum* after 8 days of treatment.

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