

12R-lipoxygenase activity is reduced in photodamaged facial stratum corneum. A novel activity assay indicates a key function in corneocyte maturation

D. Guneri* , R. Voegeli† , M. R. Munday*, M. E. Lane* and A. V. Rawlings*,‡

*UCL School of Pharmacy, London, UK, †DSM Nutritional Products Ltd, Kaiseraugst, Switzerland and ‡AVR Consulting Ltd, Northwich, UK

Received 22 February 2019, Accepted 11 April 2019

Keywords: genomics/proteomics/ELISA/cell culture, moisturization, sensitive, skin physiology/structure, skin/inflammation/allergy, UV/VIS/Fluo/CD spectroscopy

Abstract

BACKGROUND: During the late stage of keratinocyte differentiation, corneocytes gain a strong protein–lipid structure: the corneocyte envelopes (CE), composed of the inner corneocyte protein envelope (CPE) and the outer corneocyte lipid envelope (CLE). The hydrophobicity of CEs depends on the covalent attachment of linoleoyl-acyl-ceramides by transglutaminases (TG). These ceramides are processed by a range of other enzymes, including 12R-lipoxygenase (12R-LOX), before the covalent attachment of the free ω -hydroxyceramides to the CPE surface to form the CLE. The mechanical strength of CE is obtained with the formation of isodipeptide bonds by TG. The increase in hydrophobicity and rigidity leads to CE maturation which supports the integrity and mechanical resistance of the stratum corneum (SC).

OBJECTIVES: The aim of this work was to develop and validate a novel enzyme activity assay for 12R-LOX in tape strippings of photo-exposed (PE) cheek and photo-protected (PP) post-auricular SC of healthy Chinese volunteers ($n = 12$; age 25 ± 3 years).

RESULTS: A fluorescence-based assay was developed with ethyl linoleic acid as the substrate and a polyclonal antibody against 12R-LOX as an inhibitor. The specificity was shown by the lack of effect by a LOX inhibitor (ML351) and an epidermal-type lipoxygenase 3 (eLOX3) antibody on the acquired 12R-LOX activity. Reduced 12R-LOX activity was observed in the outer compared to the inner SC layers. Moreover, dramatically lower activity was shown in the PE vs. PP samples. Furthermore, the enzyme activity has a positive correlation ($r = 0.94 \pm 0.03$) with CE maturity, in particular hydrophobicity, and a negative correlation ($r = -0.96 \pm 0.01$) with transepidermal water loss (TEWL).

CONCLUSION: This novel enzyme assay revealed a lower 12R-LOX activity in tape strippings from PE cheek for the first time. This finding is in line with less mature CEs and higher TEWL compared to PP post-auricular samples. This study indicates a strong link between 12R-LOX activity and CE maturation and SC integrity.

Résumé

CONTEXTE: Pendant le stade avancé de différenciation des kératinocytes, les cornéocytes acquièrent une solide structure protéines–

lipides : l'enveloppe des cornéocytes (EC) composée de l'enveloppe protéinique des cornéocytes (EPC) interne et de l'enveloppe lipidique des cornéocytes (ELC) externe. L'hydrophobicité des EC dépend de la liaison covalente des linoléoyl-acyle-céramides par transglutaminases (TG). Ces céramides sont traités par un ensemble d'autres enzymes, y compris la 12R-lipoxygénase (12R-LOX), avant la liaison covalente des xhydroxycéramides à la surface de l'EPC pour former l'ELC. La force mécanique de l'EC est obtenue par la formation de liaisons isodipeptides par TG. L'augmentation de hydrophobicité et de la rigidité conduit à une maturation de l'EC qui soutient l'intégrité et la résistance mécanique de la couche cornée (stratum corneum, SC).

OBJECTIFS: L'objectif de ce travail était de développer et de valider un test novateur de l'activité enzymatique pour le 12R-LOX par arrachage par bande sur une joue exposée à la lumière (photo-exposed, PE) et sur de la SC à l'arrière de l'oreille protégée de la lumière (photo-protected, PP) de volontaires sains chinois ($n = 12$; $25 \text{ ans} \pm 3 \text{ ans}$).

RÉSULTATS: Un test de fluorescence a été développé avec de l'acide linoléique éthylique en tant que substrat et un anticorps polyclonal anti-12R-LOX en tant qu'inhibiteur. La spécificité a été démontrée par le manque d'effet d'un inhibiteur de LOX (ML351) et d'un anticorps anti-lipoxygénase 3 (eLOX3) de type épidermique sur l'activité du 12R-LOX acquise. Une réduction de l'activité du 12R-LOX a été observée dans les couches de SC externes par rapport aux couches internes. En outre, une activité considérablement plus faible a été démontrée dans les échantillons PE par rapport aux échantillons PP. De plus, l'activité enzymatique a une corrélation positive ($r = 0,94 \pm 0,03$) avec la maturité de l'EC, en particulier l'hydrophobicité, et une corrélation négative ($r = -0,96 \pm 0,01$) avec une perte d'eau transépidermique (transepidermal water loss, TEWL).

CONCLUSION: Ce dosage enzymatique novateur, à partir de tape stripping sur les joues exposées à la lumière, a révélé une activité 12RLOX plus faible pour la première fois. Cette découverte est cohérente avec des EC moins matures et une TEWL plus élevée par rapport aux échantillons PP de l'arrière de l'oreille. Cette étude indique un lien solide entre l'activité du 12R-LOX et la maturation de l'EC et l'intégrité de la SC.

Introduction

The skin's uppermost layer, the stratum corneum (SC), has been described as a brick-and-mortar organization thereby providing a

Correspondence: Dilek Guneri, UCL School of Pharmacy, London, UK. Tel.: +44 207 7535821; fax: +44 2077535942; e-mail: d_gueneri@yahoo.de

barrier function against dehydration and environmental impacts. In this analogy, corneocytes represent the ‘bricks’ which are embedded in intercellular lipid layers, the ‘mortar’, and hence, the mechanical strength of corneocytes is a key element in the skin barrier integrity and functions [1]. Corneocytes remain in their former keratinocyte architecture with the aggregation of keratin and the consistent crosslinking of structural proteins in the internal part of the cell membrane to create the insoluble corneocyte protein envelope [2]. Meanwhile, phospholipases degrade cell membrane phospholipids of keratinocytes and lamellar bodies to generate free fatty acids supporting the acidification of the SC [3]. The covalent attachment of ω -hydroxyceramides to the corneocyte envelopes (CE) external surface form the corneocyte lipid envelope

(CLE) then proceeds first through a peroxidation reaction through the action of 12R-lipoxygenase (12R-LOX) [4, 5]. The crosslinking of proteins and the attachment of lipids are mediated then by transglutaminases (TGs) [6]. These two processes contribute to the gain in rigidity and hydrophobicity of CEs defining their maturity [7–10]. The maturity of corneocytes contribute to the integrity [11] and cohesion [12] of the SC in order to support its function [13]. The presence of mature CEs and the intercellular lamellar lipids contributes to a healthy skin barrier functioning [2]. The ω -hydroxyceramides result from the catalytic action of 12R-LOX on esterified ω -hydroxyacyl-ceramides (CER EOS) at its linoleic acid domain which gets further processed by other enzymes such as epidermal LOX-3 (eLOX3) and other hydrolases [14–18]. Mutations in 12R-LOX have been associated with autosomal recessive congenital ichthyosis in humans [19]. A knock-out model has shown that homozygous mutant mice lack a skin barrier to an extent that post-natal death occurred within 5 h because of severe dehydration [5]. Also, the CLE has been proposed to originate from lamellar-body limiting membranes [20]. More recently, mass spectrometry has revealed low 12R-LOX protein levels in photodamaged facial SC [21] which is associated with a thinner SC and reduced CE maturation [9]. It is likely that facial SC has reduced acyl-ceramide linoleate levels that contribute to this reduced CE maturation [22, 23], and age-related reductions in covalently bound ceramides have been reported [24]. However, the activity of 12R-LOX has not been determined in these conditions.

Our aim was the establishment of a novel enzyme assay for 12R-LOX in order to understand its relationship with facial photodamage. Little is known about the levels of 12R-LOX in facial SC except the study of Voegeli *et al.* [21], and there is no detail on its activity as there are no specific inhibitors, and only a few antibodies have been described. Nevertheless, using ethyl linoleate as the substrate we were able to demonstrate specificity utilizing a 12R-LOX antibody and showed changes in 12R-LOX activity in tape strippings of photo-damaged and non-photodamaged facial skin for the first time.

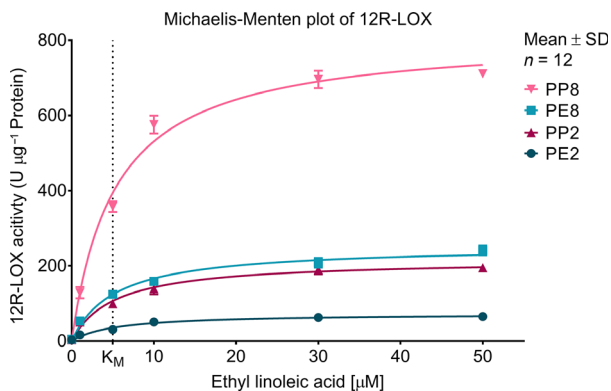


Figure 1 Determination of Michaelis constant of 12R-lipoxygenase (12R-LOX) in photo-exposed (PE) cheek (tape 2 and 8) and photo-protected (PP) post-auricular (tapes 2 and 8) sites using ethyl linoleic acid as the substrate.

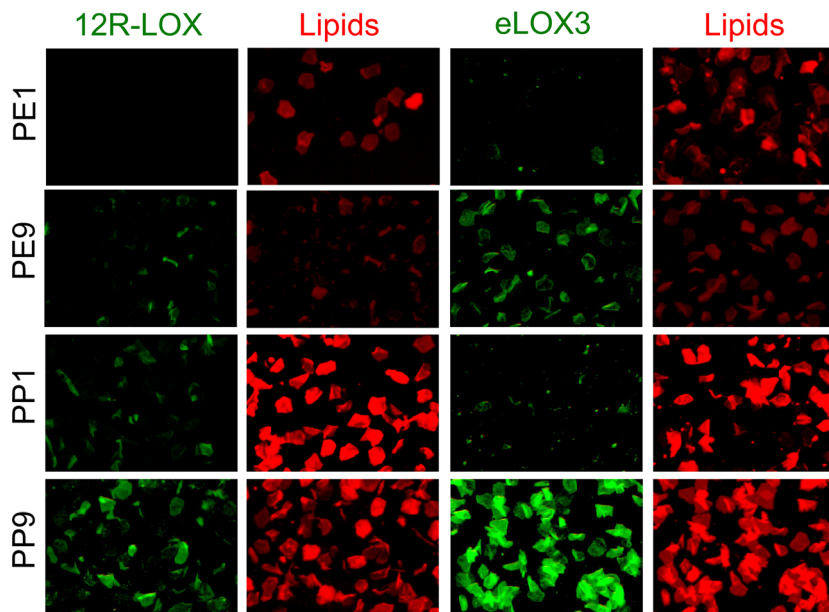


Figure 2 12R-LOX and eLOX3 immunostaining of CEs from the first and ninth tape stripping of PE Cheek and PP post-auricular sites with Nile red staining to visualize CLE. Scale bar = 100 µm.

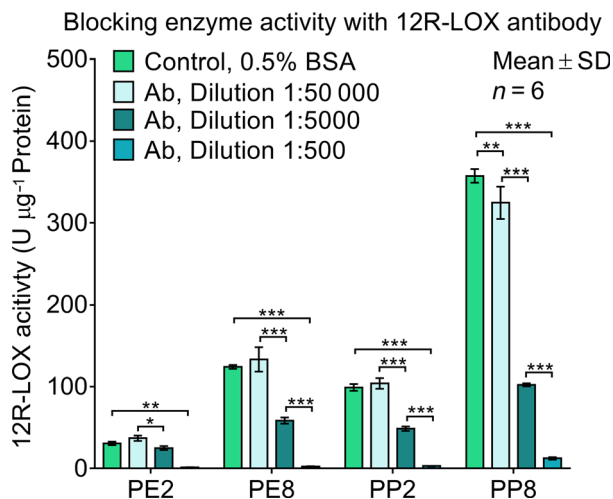


Figure 3 12R-LOX activity was blocked in PE cheek and PP post-auricular samples of the second and eighth tape strippings with a polyclonal 12R-LOX antibody. About 0.5% BSA was used as the control. Data are shown as mean \pm SD, $n = 6$ * $P \leq 0.05$, ** $P \leq 0.01$, or *** $P \leq 0.0001$.

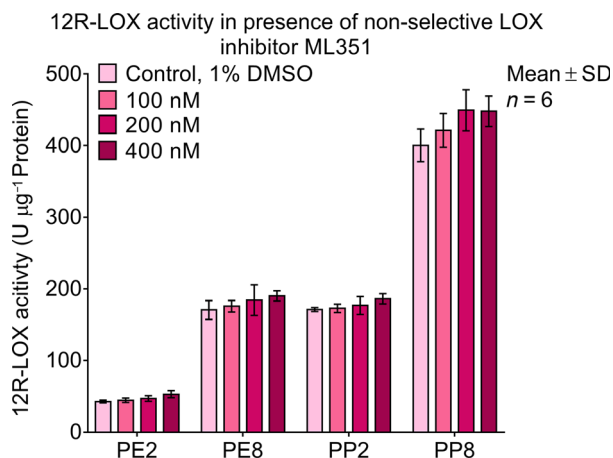


Figure 4 12R-LOX activity in PE cheek and PP post-auricular samples was not affected by the unspecific LOX inhibitor ML351. 1% DMSO was used as the control. Data are shown as mean \pm SD, $n = 6$. No significant differences were found.

Materials and methods

Study design

Ethical approval for this study was received by UCL's Research Committee and the NHS London-Bromley Research Committee (Reference: 16/LO/1672). Twelve healthy Chinese subjects (six females and males each, 26 ± 3 years old, median age 25 years) were recruited and signed the written informed consent before the study. The participants were not allowed to apply any cosmetic product on the face for at least 15 days prior to SC sampling.

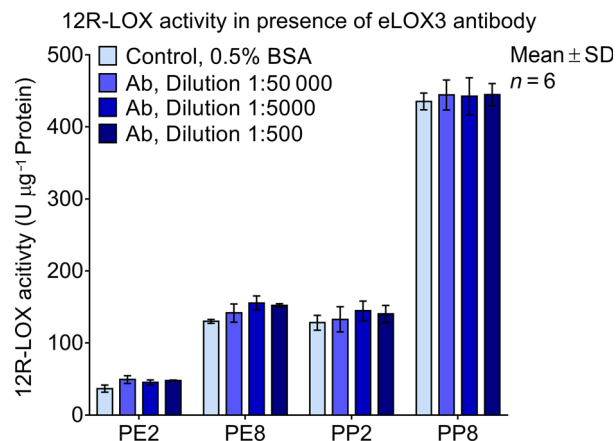


Figure 5 12R-LOX activity was not blocked by the polyclonal eLOX3 antibody. 0.5% BSA was used the control. Data are shown as mean \pm SD, $n = 6$. No significant differences were found.

SC sampling

Nine consecutive standard D-Squame[®] tapes (diameter: 2.2 cm, area: 3.8 cm²) (CuDerm Corporation, Dallas, TX, U.S.A.) were taken from the photo-exposed (PE) cheek (3 cm under the outer edge of the eye) and the photo-protected (PP) post-auricular sites (behind the earlobe). Tape strippings were performed on acclimatized skin for 20 min ($19 \pm 2^\circ\text{C}$; $44 \pm 7\%$ RH) using a pressure device (CuDerm Cooperation) applying 225 g cm^{-2} pressure on the tape for 5 s with intervals of 20 ± 5 s between each tape stripping which were removed in a single movement [25]. SC protein content was measured via infrared densitometry using a SquameScan[™]850A (Heiland Electronics GmbH, Wetzlar, Germany) [26]. Transepidermal water loss (TEWL) was measured at baseline and 30 ± 5 s after the third, sixth and ninth tape strippings to ensure consistent SC integrity (Aquaflux AF102; Biox Systems Ltd, London, U.K.). The first and ninth tapes were used to analyse the relative CE maturity (RCM) [9] while the second and eighth tapes were allocated for the 12R-LOX enzyme assay.

Immunostaining

Corneocyte envelopes were isolated with $750 \mu\text{L}$ dissociation buffer containing 20 mmol L^{-1} Tris-HCl pH 8.0, 5 mmol L^{-1} EDTA, 2% SDS, and 10 mmol L^{-1} DL-dithiothreitol for 15 min at 75°C , shaking at 600 rpm (Eppendorf, Stevenage, U.K.). CE pellets were obtained by centrifugation at $5000 g$ for 10 min and suspended in $50 \mu\text{L}$ phosphate-buffered saline (PBS) buffer (Thermo Fisher Scientific, Hertfordshire, U.K.). Triplet aliquots of $5 \mu\text{L}$ CE suspension were placed onto polysine-coated microscope slides (VWR International Ltd, Leicestershire, U.K.). A primary polyclonal antibody (1 : 25; rabbit anti-human 12R-LOX, 0.32 mg mL^{-1} ; Thermo Fisher Scientific; Cat# PA5-23608, RRID:AB_2541108) was incubated for 4 h at room temperature followed by washing three times with PBS for 5 min before incubating with the secondary antibody (1 : 100; FITC-coupled mouse anti-rabbit IgG, H&L chain; ABCAM PLC, Cambridge, U.K.) for 1 h at room temperature (in the dark). Slides were washed with PBS (three times for 5 min) and mounted with $10 \mu\text{g mL}^{-1}$ Nile red as a control (Sigma-Aldrich, Dorset,

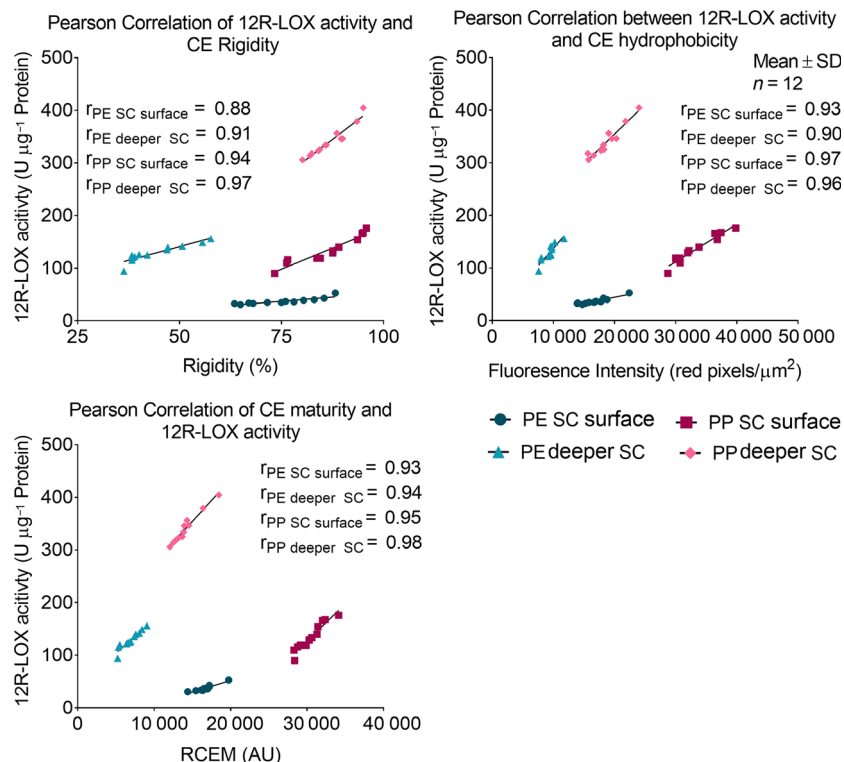


Figure 6 Pearson's correlation of 12R-LOX activity and the CE rigidity and hydrophobicity that indicate relative CE maturity (RCEM) were strongly positive in SC surface and deeper SC layers of both anatomical sites.

U.K.) in 75% glycerol solution. An additional staining method was prepared with the same approach for eLOX3 (1 : 25; rabbit anti-human e-LOX3, Biorbyt Cat# orb41336, RRID:AB_10998919) with 1 : 100 secondary antibody FITC-coupled mouse anti-rabbit (ABCAM PLC, Cambridge, U.K.).

12-LOX assay

The assay was adapted from Singh and colleagues' approach to detect 5-LOX, 12-LOX and 15-LOX activities [27]. Each tape was incubated with 0.1% Tergitol™-NP40 in 50 mmol L⁻¹ Tris (Sigma-Aldrich) with Pierce™ Protease Inhibitor Tablets (1 per 10 mL; Thermo Fisher Scientific) for 20 min on a shaker with 600 rpm at 4°C. The reaction buffer was composed of 50 mmol L⁻¹ Tris, 4 mmol L⁻¹ CaCl₂, 4 mmol L⁻¹ EDTA, 0–50 μmol L⁻¹ ethyl linoleic acid (Sigma-Aldrich), 5 μmol L⁻¹ ATP (New England Biolabs, Hitchin, U.K.) and 5 μmol L⁻¹ 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA) (Thermo Fisher Scientific). H₂DCFDA is non-fluorogenic agent that is converted in presence of oxidation into the fluorescent 2',7'-dichlorofluorescein which is widely used as indicator for reactive oxygen species. Each component was purged with argon before preparing the reaction buffer. This assay was done with a 50 μL sample and purged 150 μL reaction buffer and measured in a Corning® clear flat bottom black 96-well plate (Sigma-Aldrich) with an interval of 1 min for 20 min at 37°C with Ex/Em: 495/527 nm in SpectraMax iD3 (Molecular Devices, Wokingham, U.K.). The anti-12R-LOX and eLOX3 polyclonal antibodies used in the immunostaining were diluted in purged 0.5% bovine serum albumin (BSA) in reaction buffer as well as 5-(methylamino)-2-(1-naphthalenyl)-4-oxazolecarbonitrile (ML351) (Sigma-Aldrich) as a blocker for the LOX enzymes. 12R-LOX activity was expressed as U min⁻¹ normalized to the total SC protein on tape strips.

Statistics

All statistical analysis was performed with GRAPHPAD PRISM (version 7). The data passed the D'Agostino & Pearson's normality test; therefore, one-way ANOVA with Sidak–Holm *post-hoc* test was chosen to

Pearson Correlation between SC Integrity and 12R-LOX activity

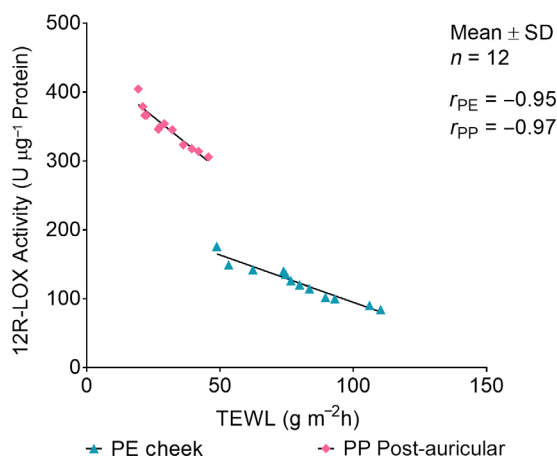


Figure 7 Pearson's correlation of SC integrity after nine tape strippings and the 12R-LOX activity in the deeper SC layers (tape 8) show a clear negative relationship.

determine statistical differences. Results for each set of experiments are shown as the mean \pm SD ($n = 6$ or 12) while statistical significance is represented as $*P \leq 0.05$, $**P \leq 0.01$, or $***P \leq 0.001$.

Results

The principle of this assay is based on the oxidation of the fluorogenic agent (H_2DCFDA) by the reactive oxygen species created during the LOX reaction. Buffer-extracted 12R-LOX activity was determined with ethyl linoleic acid in order to set-up a Michaelis-Menten plot to characterize the obtained activity from the second and eighth tapes of PE cheek and PP post-auricular samples. All four samples reached their K_m value at $5.23 \pm 0.07 \mu\text{mol L}^{-1}$; however, V_{max} was distinctive between both anatomical sites and depths (Fig. 1). The 12R-LOX activity reached 3x higher V_{max} values in the PP post-auricular sites at the surface and the deeper SC layer compared to PE cheek samples.

Clear individual staining of 12R-LOX and eLOX3 positive CEs with a higher intensity in the PP post-auricular samples were immunohistochemically shown (Fig. 2). Thus, the selected antibodies can be used for the development of the 12R-LOX enzyme assay.

The 12R-LOX antibody was used to demonstrate the concentration-dependent decrease in the 12R-LOX activity. While the 1 : 5000 dilution showed a half-maximal ($***P < 0.001$) and the 1 : 500 dilution a full enzyme inhibition ($***P < 0.001$), the control (0.5% BSA) did not impact the activity (Fig. 3). Moreover, the same approach was performed using a LOX inhibitor (ML351) which also did not influence the 12R-LOX activity (Fig. 4). A proof of concept experiment was performed with the antibody for eLOX3 that was used in the immunostaining (Fig. 2) with the same set-up as for the 12R-LOX activity assay (Fig. 3). This assay showed no difference in 12R-LOX activity in the presence of eLOX3 antibody (Fig. 5) in contrast to the results obtained in the presence of 12R-LOX antibody.

Pearson's correlation test was performed between CE rigidity and hydrophobicity as well as RCEM of the first and ninth tape strippings and the obtained 12R-LOX activity PE cheek and PP post-auricular samples from the second and eighth tape strippings. This revealed a positive correlation for PE cheek and PP post-auricular maturation and the enzyme activity of 12R-LOX (Fig. 6). The TEWL upon tape stripping can be used to reflect the SC integrity of the PE cheek and PP post-auricular sites which was correlated to their corresponding 12R-LOX activity. This demonstrated a clear negative correlation where high TEWL is associated with low 12R-LOX activity (Fig. 7).

Discussion

The importance of essential fatty acid-containing lipids for skin physiology was highlighted from the early work of Burr & Burr (1930) [28]. Later, it was demonstrated that these benefits were derived from lipoxygenase-catalysed conversion of linoleoyl-acyl-ceramides [29, 30]. The importance of these metabolic products, their formation and role in CLE formation was highlighted by the work of Brash and collaborators who focused on 12R-LOX as the first enzyme in ceramide EOS linoleate oxidation [14–17]. However, we were interested in this enzyme because of the known reduced maturation of facial SC corneocytes [9, 10, 31, 32] as reduced mass levels of 12R-LOX were recently reported in Caucasian facial SC [21]. This finding was accompanied with lower 12R-LOX mass levels in Albino Africans compared to Black Africans and Caucasians [33].

This work is the first measurement of 12R-LOX activity obtained from facial SC tape strippings based on an assay from a previously

published fluorogenic LOX method [27]. This subtype of the LOX enzymes has only recently attracted attention from skin scientists and, therefore, specific substrates and inhibitors are yet not fully commercialized. Ethyl linoleic acid was chosen as the substrate as it resembles the esterified linoleic acid domain in CER EOS which is the natural substrate of 12R-LOX. The enzyme activity showed the same K_m value ($5.23 \pm 0.07 \mu\text{mol L}^{-1}$) in all tested samples. Samples from less mature deeper SC layers (tape 8) of both anatomical sites had a higher enzyme activity than those from more mature superficial layers (tape 2); however, samples taken from the PP post-auricular test site have a higher enzyme activity compared to PE cheek samples. The obtained fluorescence had to be validated to be certain that it originates from 12R-LOX activity. Direct methods in terms of activation or inhibition are not yet available but antibodies are natural glycoproteins that can detect and bind a specific antigen which was chosen as a suitable blocking agent in this experimental set-up. The immunostaining showed a clear fluorescence signal on CEs for both, 12R-LOX and eLOX3, which was proof of antigen detection. A general unspecific LOX inhibitor (ML351) was used to exclude 5-LOX, 12-LOX and 15-LOX activities. The presence of the eLOX3 antibody was ineffective in reducing the detected fluorescence which revealed that the inhibition by 12R-LOX antibody is not owing to an unspecific spatial blockage by the antibody but an antigen-specific blocking of 12R-LOX. A parallel study investigated the impact of 12R-LOX antibody in *ex vivo* CE maturation showing that CE rigidity improved while CE hydrophobicity remained unchanged in all tested samples (data not shown). The lower 12R-LOX activity in PE samples is consistent with the reduced 12R-LOX protein mass levels in PE cheek compared to PP post-auricular sites [21] as well as reduced CE maturation on this site [9, 10, 31, 32]. Decreased 12R-LOX activity and reduced corneocyte maturation *ex vivo* demonstrate the importance of this enzyme for the SC maturation by influencing the CE hydrophobicity (D. Guneri, unpublished data). Further work is ongoing to increase epidermal gene expression and enzyme activity of 12R-LOX in order to enhance CE hydrophobicity thereby contributing to the SC barrier function.

In conclusion, the importance of the hydroxylated variants of linoleoyl ceramide EOS first identified in the late 1980s [30] together with the subsequent cloning and mechanistic studies on 12R-LOX from in the late 1990s [15] onwards and the reported reduced mass levels of 12R-LOX in photodamaged facial skin [21] guided our activity-based studies on 12R-LOX. We report a novel antibody inhibition fluorescence-based assay to show depth activity profiles in the SC and reduced activity in photodamaged SC that correlates with poor CE hydrophobicity. This assay will be of use in other disorders of SC maturation.

Acknowledgements

We would like to thank Dr. Rebecca Lever for access to fluorescence microscopy facilities. We would like to express our special gratitude to all our volunteers.

Sources of funding

This study was financially supported by DSM Nutritional Products Ltd, Basel, Switzerland.

Conflicts of interests and disclosures

RV is an employee of DSM Nutritional Products Ltd. AVR is a consultant to DSM Nutritional Products Ltd.

References

- Nemes, Z. and Steinert, P.M. Bricks and mortar of the epidermal barrier. *Exp. Mol. Med.* **31**, 5–19 (1999).
- Candi, E., Schmidt, R. and Melino, G. The cornified envelope: a model of cell death in the skin. *Nat. Rev. Mol. Cell Biol.* **6**, 328–340 (2005).
- Fluhr, J.W., Kao, J., Ahn, S.K., Feingold, K.R., Elias, P.M. and Jain, M. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *J. Invest. Dermatol.* **117**, 44–51 (2001).
- Wertz, P.W. and van den Bergh, B. The physical, chemical and functional properties of lipids in the skin and other biological barriers. *Chem. Phys. Lipids* **91**, 85–96 (1998).
- Epp, N., Fürstenberger, G., Müller, K. et al. 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J. Cell Biol.* **177**, 173–182 (2007).
- Nemes, Z., Marekov, L.N., Fesus, L. and Steinert, P.M. A novel function for transglutaminase 1: attachment of long-chain -hydroxyceramides to involucrin by ester bond formation. *Proc. Natl Acad. Sci.* **96**, 8402–8407 (1999).
- Michel, S., Schmidt, R., Shroot, B. and Reichert, U. Morphological and biochemical characterization of the cornified envelopes from human epidermal keratinocytes of different origin. *J. Invest. Dermatol.* **91**, 11–15 (1988).
- Harding, C.R., Long, S., Richardson, J., Rogers, J., Zhang, Z., Bush, A. and Rawlings, A.V. The cornified cell envelope: an important marker of stratum corneum maturation in healthy and dry skin. *Int. J. Cosmet. Sci.* **25**, 157–167 (2003).
- Gumeri, D., Voegeli, R., Gurgul, S.J., Munday, M.R., Lane, M.E. and Rawlings, A.V. A new approach to assess the effect of photodamage on corneocyte envelope maturity using combined hydrophobicity and mechanical fragility assays. *Int. J. Cosmet. Sci.* **40**, 207–216 (2018).
- Hirao, T. Involvement of transglutaminase in ex vivo maturation of cornified envelopes in the stratum corneum. *Int. J. Cosmet. Sci.* **25**, 245–257 (2003).
- Meguro, S., Arai, Y., Masukawa, Y., Uie, K. and Tokimitsu, I. Relationship between covalently bound ceramides and transepidermal water loss (TEWL). *Arch. Dermatol. Res.* **292**, 463–468 (2000).
- Wertz, P.W., Swartzendruber, D.C., Kitko, D.J., Madison, K.C. and Downing, D.T. The role of the corneocyte lipid envelopes in cohesion of the stratum corneum. *J. Invest. Dermatol.* **93**, 169–172 (1989).
- Rawlings, A. Sources and role of stratum corneum hydration. In: *Skin Barrier* (Elias, P. and Feingold, K. eds.), pp. 399–425. CRC Press, New York (2005).
- Chiba, T., Thomas, C.P., Calcutt, M.W., Boeglin, W.E., O'Donnell, V.B. and Brash, A.R. The precise structures and stereochemistry of trihydroxy-linoleates esterified in human and porcine epidermis and their significance in skin barrier function: implication of an epoxide hydrolase in the transformation of linoleate. *J. Biol. Chem.* **291**, 14540–14554 (2016).
- Boeglin, W.E., Kim, R.B. and Brash, A.R. A 12R-lipoxygenase in human skin: mechanistic evidence, molecular cloning, and expression. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6744–6749 (1998).
- Zheng, Y., Yin, H., Boeglin, W.E., Elias, P.M., Crumrine, D., Beier, D.R. and Brash, A.R. Lipoxygenases mediate the effect of essential fatty acid in skin barrier formation: a proposed role in releasing omega-hydroxyceramide for construction of the corneocyte lipid envelope. *J. Biol. Chem.* **286**, 24046–24056 (2011).
- Zheng, Y. and Brash, A.R. Formation of a cyclopropyl epoxide via a leukotriene a synthase-related pathway in an anaerobic reaction of soybean lipoxygenase-1 with 15-hydroperoxyeicosatetraenoic acid: evidence that oxygen access is a determinant of secondary reactions with fatty acid hydroperoxides. *J. Biol. Chem.* **285**, 13427–13436 (2010).
- Yamanashi, H., Boeglin, W.E., Morisseau, C., Davis, R.W., Sulikowski, G.A., Hammock, B.D. and Brash, A.R. Catalytic activities of mammalian epoxide hydrolases with *cis* and *trans* fatty acid epoxides relevant to skin barrier function. *J. Lipid Res.* **59**, 684–695 (2018).
- Eckl, K.-M., Krieg, P., Küster, W. et al. Mutation spectrum and functional analysis of epidermis-type lipoxygenases in patients with autosomal recessive congenital ichthyosis. *Hum. Mutat.* **26**, 351–361 (2005).
- Crumrine, D., Khnykin, D., Krieg, P. et al. Perspective: Mutations in recessive congenital ichthyoses illuminate the origin and functions of the corneocyte lipid envelope. *J. Invest. Dermatol.* **139**, 760–768 (2018).
- Voegeli, R., Monneuse, J.-M., Schoop, R., Summers, B. and Rawlings, A.V. The effect of photodamage on the female Caucasian facial stratum corneum corneome using mass spectrometry-based proteomics. *Int. J. Cosmet. Sci.* **39**, 637–652 (2017).
- Rogers, J., Harding, C., Mayo, A., Banks, J. and Rawlings, A. Stratum corneum lipids: the effect of ageing and the seasons. *Arch. Dermatol. Res.* **288**, 765–770 (1996).
- Ishikawa, J., Yoshida, H., Ito, S. et al. Dry skin in the winter is related to the ceramide profile in the stratum corneum and can be improved by treatment with a *Eucalyptus* extract. *J. Cosmet. Dermatol.* **12**, 3–11 (2013).
- Fujiwara, A., Morifuji, M., Kitade, M. et al. Age-related and seasonal changes in covalently bound ceramide content in forearm stratum corneum of Japanese subjects: determination of molecular species of ceramides. *Arch. Dermatol. Res.* **310**, 729–735 (2018).
- Breternitz, M., Flach, M., Präßler, J., Elsner, P. and Fluhr, J.W. Acute barrier disruption by adhesive tapes is influenced by pressure, time and anatomical location: integrity and cohesion assessed by sequential tape stripping; a randomized, controlled study. *Br. J. Dermatol.* **156**, 231–240 (2007).
- Voegeli, R., Heiland, J., Doppler, S., Rawlings, A.V. and Schreier, T. Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry. *Skin Res. Technol.* **13**, 242–251 (2007).
- Singh, R.K., Gupta, S., Tiwari, P. et al. A fluorescent based enzyme assay for recombinant human lipoxygenase enzyme isoforms. *Pharmacologia* **3**, 387–396 (2012).
- Burr, G.O. and Burr, M.M. On the nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* **86**, 587–621 (1930).
- Bowser, P.A., Nugteren, D.H., White, R.J., Houtsmuller, U.M.T. and Prottey, C. Identification, isolation and characterization of epidermal lipids containing linoleic acid. *Biochim. Biophys. Acta BBA - Lipids Lipid Metab.* **834**, 419–428 (1985).
- Nugteren, D.H., Christ-Hazelhof, E., van der Beek, A. and Houtsmuller, U.M. Metabolism of linoleic acid and other essential fatty acids in the epidermis of the rat. *Biochim. Biophys. Acta* **834**, 429–436 (1985).
- Mohammed, D., Matts, P.J., Hadgraft, J. and Lane, M.E. Depth profiling of stratum corneum biophysical and molecular properties: depth profiling of stratum corneum. *Br. J. Dermatol.* **164**, 957–965 (2011).

32. Raj, N., Voegeli, R., Rawlings, A.V., Summers, B., Munday, M.R. and Lane, M.E. Variation in the activities of late stage flaggrin processing enzymes, calpain-1 and bleomycin hydrolase, together with pyrrolidone carboxylic acid levels, corneocyte phenotypes and plasmin activities in non-sun-exposed and sun-exposed facial stratum cor. *Int. J. Cosmet. Sci.* **38**, 567–575 (2016).
33. Voegeli, R., Monneuse, J.-M., Klose, C., Schoop, R., Summers, B., Rudolph, T. and Rawlings, A.V. Phenotypic changes in the corneome and ceramidome of photodamaged dry facial stratum corneum from different ethnic groups. Presented at the International Federation of Societies of Cosmetic Chemists in Munich, Germany (2018).