ORIGINAL ARTICLE

In vitro and *in vivo* studies with tetra-hydro-jasmonic acid (LR2412) reveal its potential to correct signs of skin ageing

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Abstract

Background LR2412, a synthetic derivative of jasmonic acid, improved the reconstruction and homeostasis of our organotypic skin models.

Objectives The need for efficient 'anti-ageing' treatments, in particular for the management of photoaged skin, prompted us to investigate this new ingredient for its potential to correct signs of skin ageing *in vitro* and *in vivo* and to identify its mode of action.

Results *In vitro*, penetration of LR2412 was evaluated using a Franz diffusion cell on excised human skin. Its exfoliating properties and interactions with the stratum corneum were studied using electron microscopy and X-ray diffraction. Experiments were performed on a human reconstructed skin model. *In vivo*, the effects of LR2412 on steroid-induced skin atrophy, a clinical skin ageing model, were assessed vs. vehicle. A patch test study evaluated its effect on deposition of fibrillin-rich microfibrils in the papillary dermis in clinically photoaged volunteers. A clinical study on the appearance of crow's feet wrinkles was conducted over 3 months of daily application. Penetration studies revealed that LR2412 reaches viable epidermis and superficial dermis, which are skin targets of anti-ageing actives. Within the upper layers of the stratum corneum LR2412 accelerates desquamation and improves the mechanical properties. At the dermal–epidermal junction of reconstructed skin, collagen IV, laminin-5 and fibrillin were stimulated. *In vivo*, LR2412 reversed steroid-induced atrophy. The patch test model confirms the deposition of fibrillin-rich microfibrils, then an in use clinical study revealed that it reduced facial wrinkles.

Conclusions The *in vitro* and *in vivo* data demonstrate that based on its multiple interactions within human skin, LR2412 has potential to partially correct the signs of ageing in intrinsically and photoaged skin.

Received: 23 August 2012; Accepted: 16 January 2013

Conflict of Interest

C. Tran, J.F. Michelet, L. Simonetti, F. Fiat, A. Garrigues, A. Potter, E. Segot and O. de Lacharrière are employees of L'Oreal. C.E.M. Griffiths has received speakers' fees from L'Oreal.

Funding sources

The patch test study conducted herein was funded by a grant from L'Oreal R&D.

Introduction

Like all organs, skin is subject to intrinsic ageing. It is also directly exposed to the environment, thus making it particularly vulnerable to extrinsic, in particular ultraviolet radiation (UV)-induced, damage which accelerates the ageing process known as photoageing. The epidermis becomes atrophied[1] and the structure of the dermal–epidermal junction (DEJ) and the dermal extracellular matrix (ECM) are altered, resulting in loss of dermal papillae.[2–4] Certain of the agents used to improve the appearance of photoaged skin have inherent side effects, e.g. irritation, teratogenicity.[5]

We have identified a novel substance which may be reparative of photoaged skin. Jasmonic acid, a plant hormone derived from linolenic acid, is known to be involved in plant stress regulation, wound repair and defence.[6] Using green chemistry,[7] we have synthesized analogues of jasmonic acid among which 20 were selected for further investigation. Following a thorough safety analysis (irritancy, sensitization, mutagenesis, clastogenesis and teratogenesis), these selected analogues were pre-evaluated in human reconstructed skin models regarding their capacity to sustain epidermal homeostasis. LR2412, a derivative with amphiphilic properties, showed the most promising

results and was selected for further *in vitro* and *in vivo* investigations.

Methods

Penetration studies

The study was conducted according to the OECD[8] and SCCS guidelines[9] on excised human skin from abdominal plastic surgery. This model has been shown to be predictive of *in vivo* results.[10,11] Briefly, the study was performed using a static Franz-type diffusion cell. A precise quantity (2mg/cm^2) of formulated LR2412 (4%, w/v) was applied topically for 24 h. At the end of exposure time, the skin surface was washed twice with 0.6 mL of a 5% Lauryl ether sulfate solution. LR2412 was assessed in the *stratum corneum*, epidermis, superficial dermis and receptor fluid by chromatographic analyses using a HP1200 series system (Waldbronn, Germany) interfaced with a API3200™ LC–MS/MS system (Applied Biosystems, Foster City, CA, USA) by a Turbo V™ interface equipped with an ESI probe.

X-ray diffraction

X-ray diffraction was used for analysing lipid organization at the molecular level. To assess the effect of LR2412 on the human *stratum corneum*, we performed combined small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) experiments, with a micrometric spatial resolution. Experiments were performed at the European Synchrotron Radiation Facility (Grenoble, France) at the microfocus beam line ID13.[12] Single sheets of human *stratum corneum* samples were obtained and kept in physiological conditions (samples were neither frozen nor stained). Thin strips were cut with a razor blade ($\approx 0.1 \times 10$ mm^2). Both ends were then placed on two horizontal notches 3 mm apart so that the incident beam of X-rays was parallel to the plane of the *stratum corneum*.

Two types of sample were analysed at 22°C and 35% relative humidity: untreated (control) and after treatment with LR2412 (2%; $n = 2$; with triplicate repeats).

Desquamation

Desquamation was evaluated by counting released corneocytes following incubation of discs of 4 mm diameter punched out of abdominal skin and placed in a 96-well plate. A 2% (w/v) solution of LR2412 was prepared in a PBS buffer supplemented with 0.1% Triton X100 (pH 7.4) and 50 µL added to each well (control: PBS buffer, 0.1% Triton X100). Incubation was carried out at 37°C with continuous stirring for 24 h. Thereafter, 10 µL were placed in a Malassez cell (hematimeter) to count released corneocytes.

Transmission electronic microscopy

Skin biopsies from abdominal plastic surgery were treated for 18 h with either LR2412 (2%, w/v in PBS) or with PBS alone before fixation in Karnovsky fixative solution (0.1 mol/L cacodylate buffer, post-fixed in 1% (w/v) osmium tetroxide).[13] Samples were dehydrated in a graded series of ethanol then embedded in Epon 812 resin. Ultrathin sections (80 nm) were double stained with uranyl acetate and lead citrate. The ultrastructure of the *stratum corneum* was observed in a Zeiss EM C902 microscope (Zeiss Gmbh, Jena, Germany).

Reconstructed human skin

REALSKIN® samples (Episkin SNC, Lyon, France), a skin equivalent composed of a living stratified epidermis on a fibroblast populated collagen dermal equivalent, were incubated with fresh medium for 5 days at 37°C under 5% CO_2 with 10 and 100 µM (0.0002% and 0.002%, respectively) LR2412 added to culture medium vs. medium alone (the media were renewed every 48 h). Skin equivalents were used for immunohistochemical analysis. Samples were embedded in optimal cutting temperature compound (Tissue Tek®; Miles, Naperville, IL, USA) and snap frozen in liquid nitrogen. Immunofluorescence staining was performed on five cryosections per sample (7 µm). A panel of ECM proteins were identified including: collagen IV (clone M0785; Dako, Trappes, France) diluted 1 : 50; laminin-5 (clone mAb19562; Millipore, Molsheim, France) diluted 1 : 200 and mouse anti-human fibrillin-rich microfibrils (clone 11C1.3; Southern Biotech, Birmingham, AL, USA) diluted 1 : 100. Staining was visualized using the appropriate secondary antibody conjugated to Alexa488® (1 : 500; Invitrogen, Carlsbad, CA, USA). Nuclei were stained with propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) before examination under an Axiovert 135 fluorescent microscope (Zeiss, Satrouville, France).

Reversal of topical steroid-induced skin atrophy

A randomized, controlled pilot study was performed to evaluate the potential of LR2412 to reverse steroid-induced skin atrophy. The ethical committee of University Hospital, Cimiez, Nice, France (Ref: 09.056) and the Agence Française de Sécurité Sanitaire des Produits de Santé (AFSSAPS) (Ref: 2009-A01067-50) approved the study. Thirty-two healthy subjects between 23 and 45 years (mean age: 33 ± 7 years) were included after giving written informed consent. Corticosteroids were applied for 4 weeks to induce histological and clinical signs of skin ageing (dermatoporosis), each subject acting as their own control. Volunteers were pre-treated on their volar forearms with 2% (w/v) LR2412 O/W emulsion or its vehicle, twice daily for 4 weeks (2 mg/cm^2). Sites of application were randomized. During the next 4 weeks, they received concomitantly with their investigational products, topical 0.05% clobetasol propionate (Dermoval®; GlaxoSmithKline, Marly le Roi, France) on their forearms (2 mg/cm^2). Thereafter, 2% (w/v) LR2412 or the vehicle was applied on the forearms for 2 weeks. Clinical grading for skin atrophy was based on a 5-point scale for the skin microrelief

Table 1 Penetration results : percent of applied dose of 4% LR2412. The percent of applied dose, the amount and the concentration of LR2412 were quantified in each skin compartment. Values are the mean ± SEM of three determinations in three donors. Data are expressed as percentage of applied dose, amount of LR2412 per unit area, and concentration

Stratum corneum	Applied dose (%)	0.928 ± 0.190
	Amount (μg/cm^2)	0.762 ± 0.157
	Concentration (μmol/L)	3556 ± 690
Epidermis	Applied dose (%)	0.741 ± 0.171
	Amount (μg/cm^2)	0.606 ± 0.138
	Concentration (μmol/L)	283 ± 61
Superficial dermis	Applied dose (%)	0.266 ± 0.056
	Amount (μg/cm^2)	0.217 ± 0.046
	Concentration (μmol/L)	27.1 ± 4.1
Receptor fluid	Applied dose (%)	3.59 ± 1.04
	Amount (μg/cm^2)	2.94 ± 0.852

(from 0 = normal to 4 = severe thinning and loss of appendages) and telangectasia (from 0 = normal to 4 = evident telangectasia) was performed at baseline (D0), D28, D43, D56 (end of the corticoid application period) and D67 at CPCAD. Skin biopsies (1× 2 mm diameter punch) were fixed in 10% phosphate-buffered formaldehyde and embedded in paraffin. Sections (5 μm) were mounted onto slides and stained with haematoxylin and eosin (HE) according to standard procedures. All sections were examined under a Leica DMRB microscope and epidermal thickness measurements were performed on HE-stained slides using the IM1000 (Leica Microsystems, Wetzlar, Germany) image analyzer software.

Patch test study

Nine healthy but clinically photoaged volunteers were recruited (male: 2; female: 7; age range 41–65 years) and subjected to an extended 12-day patch test assay.[14,15] Test substances (vehicle, 2% LR2412 and 0.07% retinol; 30 μL) were applied separately to the extensor photoaged aspect of the forearm under standard 6 mm diameter Finn chambers (Scanpore, Tuulsula, Finland). As a baseline untreated control, an area was occluded for the study period. Products were applied to clean skin on days 1, 4 and 8 of the assay. All-*trans* retinoic acid provided our 'gold standard' positive control (*t*RA; 0.025%; Retin-A® cream; Janssen-Cilag Ltd, Beerse, Belgium; 30 μL); this was applied to an untreated site on day 8 of the assay and left *in situ* for 4 days to avoid potential complications of irritancy caused by extended occlusion. On day 12, all of the Finn chambers were removed and 3 mm punch biopsies were taken under 1% lignocaine local anaesthesia from each test site. Biopsies were embedded in Tissue-Tek®, snap frozen in liquid nitrogen and stored at −70°C prior to immunohistochemical analyses. The South Manchester Local Research Ethics Committee approved the study and all subjects gave written, informed consent (REC reference 09/H1004/68).

Frozen sections were prepared at a thickness of 10 μm (OTF cryostat, Bright Instruments Ltd, Cambridge, UK) and mounted onto gelatin-coated slides. Immunohistochemistry was performed as previously described[14–16] to identify a panel of ECM molecules from the 12-day patch test assay. Primary antibodies were applied overnight at 4°C. These were either: mouse anti-human fibrillin-rich microfibrils (clone 11C1.3) diluted 1 : 100; rat anti-human pro-collagen I (clone M-58; Chemicon Interna-

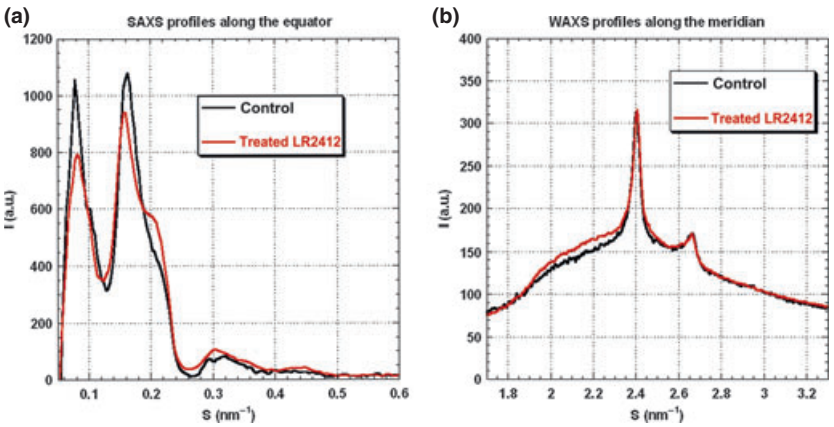

Figure 1 X-ray diffraction reveals that LR2412 incorporates into the intercellular lipids without any structural disruption of their crystalline organization. (a) Quatorial SAXS: only small differences exist between the two profiles. The positions of the two major peaks are almost identical (S = 0.08 and 0.16 nm^{-1} for the controls, and S = 0.08 and 0.17 nm^{-1} for the treated sample, respectively). The shoulder at 0.2 nm^{-1} increased after treatment, reflecting a small change in the molecular organization within the Landmann units, indicating that LR2412 incorporates into the intercellular lipids without any structural disruption (densification of lipid organization). (b) Meridional WAXS: exhibits two broad diffuse rings at 1.05 and 2.17 nm^{-1} (keratins) and two sharp arcs at 2.44 and 2.70 nm^{-1}. Intensity and position of the arcs remain unchanged after treatment, indicating that LR2412 does not disturb the crystalline organization of the intercellular lipids.

tional, Inc., Temecula, CA, USA) diluted 1 : 1000 or mouse anti-human collagen VII (clone LH7.2; Sigma Chemical Company, St Louis, MO, USA) diluted 1 : 100. Negative controls were by incubation of isotype sera at the appropriate concentration or omission of primary antibody. Sections were washed in Tris-Buffered Saline, prior to incubation with the appropriate biotinylated secondary antibody for 30 min. Antibody staining was visualized using an immunoperoxidase reaction (VectaStain® Elite ABC system; Vector Laboratories, Burlingame, CA, USA) utilizing Vector SG® as chromogen. Following light counterstaining with nuclear fast red, sections were serially dehydrated and permanently mounted. Stained sections were randomized, blinded and examined on a Nikon OPTIPHOT microscope (Tokyo, Japan). The degree of immunostaining for fibrillin-rich microfibrils and pro-collagen I were assessed as previously described.[15,16] In brief, a five point semi-quantitative scale was used where 0 = no staining and 4 = maximal staining within the experiment. Four sections (including control) were examined per subject, per site, per treatment and the average score calculated. Quantification of collagen VII staining was performed in ImageJ (National Institute of Health, Bethesda, MA, USA). Twenty-four bit RGB images were corrected for uneven illumination and split into red, green and blue channel stacks to provide 8-bit greyscale images. Images were inverted and signal across the DEJ quantified (three sites per image; three images per site). Mean data produced from image analysis were then displayed as line profiles and the area under the curve (AUC) integrated for statistical analysis.

Differences in the amount of immunostaining produced by the different formulations under test were assessed for significance using the repeated measures analysis of variance (ANOVA). Results were considered significant if $P < 0.05$ (95% confidence level) and were calculated using SPSS+ v15 software (SPSS Inc., Chicago, IL, USA).

3-month facial application

Healthy female volunteers ($n = 42$; age range 52 –65 years) were recruited for a randomized controlled pilot study. After 2 weeks of pre-treatment of the whole face with a O/W emulsion (vehicle), the same O/W formula containing 2% (w/v) LR2412 was applied on one-half of the face (randomized) and the vehicle alone on the other side for 3 months. At baseline (D0) and after 1 (D28) and 3 (D84) months of product use, assessment of crow's foot wrinkles was performed using the 'shadow method' first described by Corcuff.[17] The shadows created on the silicone replicas (Silflo®, Flexico, UK) were quantified by image analysis software (Quantirides®; Monaderm, Monaco) on the negative Silflo® replica.

Negative replicas were obtained from the two crow's feet areas (right and left side of the face) using Silflo® polymer according to a standardized procedure. Replicas were labeled and stored until the end of the study and analysed at the same time.

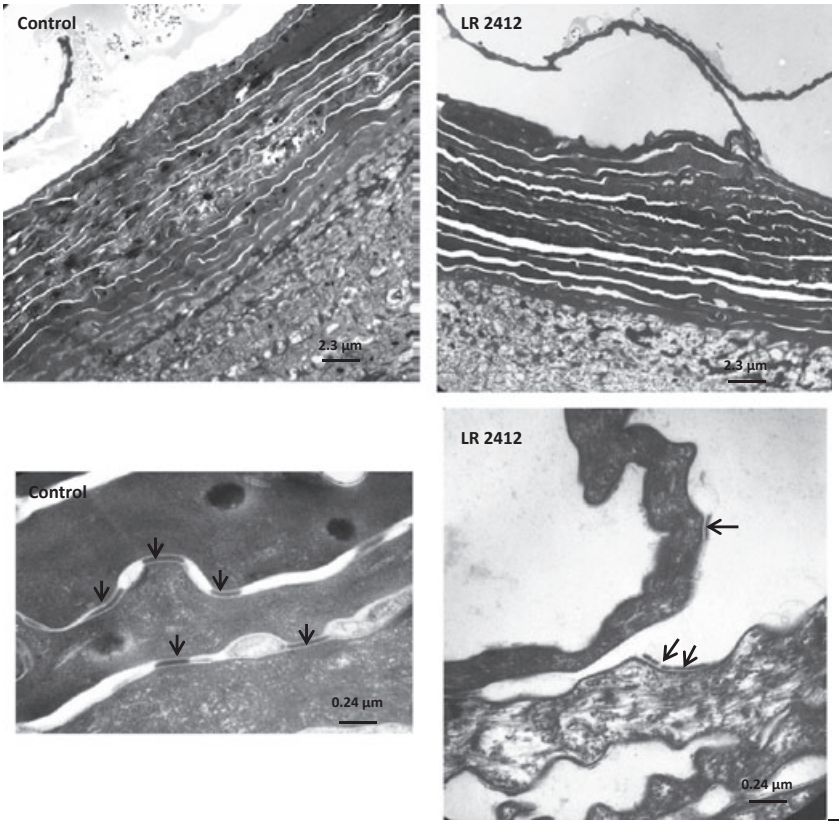

Figure 2 LR2412 induced desquamation. Transmission electron microscopy depicts the tight cohesion between corneocytes by corneodesmosomes (arrows) in the control and the loss of cohesion after 24 h exposure to 2% LR2412.

The number of wrinkles was counted and their length, depth and surface area measured and averaged. Evolution of the wrinkles over time for each treatment was assessed using an ANOVA with two factors (subject as random and time as fixed factor, respectively) followed by a Dunett test comparing D28 and D84 to D0. Data were compared at each time point using a Student's test or Wilcoxon test for paired data according to the normality of the distribution (Shapiro-Wilk test at 1%). The significant threshold for all analysis was fixed at the 95% confidence level.

Results

Penetration study

The overall recovery of LR2412 is between 91.7% and 110.1%, in respect with the criteria of the guidelines (i.e. 100 ± 15%). Evaluation of the penetration properties of LR2412 (4%) formulation applied (2 mg/cm²) onto human skin in a Franz diffusion cell showed that following 24 h of treatment 0.93% of applied LR2412 was present in the *stratum corneum*, 0.74% in the epidermis, 0.27% in the superficial dermis and 3.59% in the receptor fluid (Table 1). LR2412 concentrations in different skin layers are consistent with its biological activity. LR2412 diffuses in all skin layers with a decreasing penetration profile from the stratum corneum to superficial dermis. LR2412 reaches viable epidermis and superficial dermis, which are skin targets of anti-ageing actives.

Interaction with the *stratum corneum*

X-ray diffraction studies of isolated human *stratum corneum* showed that LR2412 was incorporated into the intercellular lipids without any structural disruption of their crystalline organization (Fig. 1). Exposure of isolated human *stratum corneum* to LR2412 (2% w/v) induced accelerated release of corneocytes. Transmission electron microscopy of normal human skin biopsies revealed that LR2412 modified the corneodesmosomal structure leading to a loss of corneocyte cohesion and eventually desquamation (Fig. 2).

Reconstructed human skin

Exposure of REALSKIN® to LR2412 (10 μmol/L) for 5 days increased the deposition of collagen IV, and laminin-5 at the

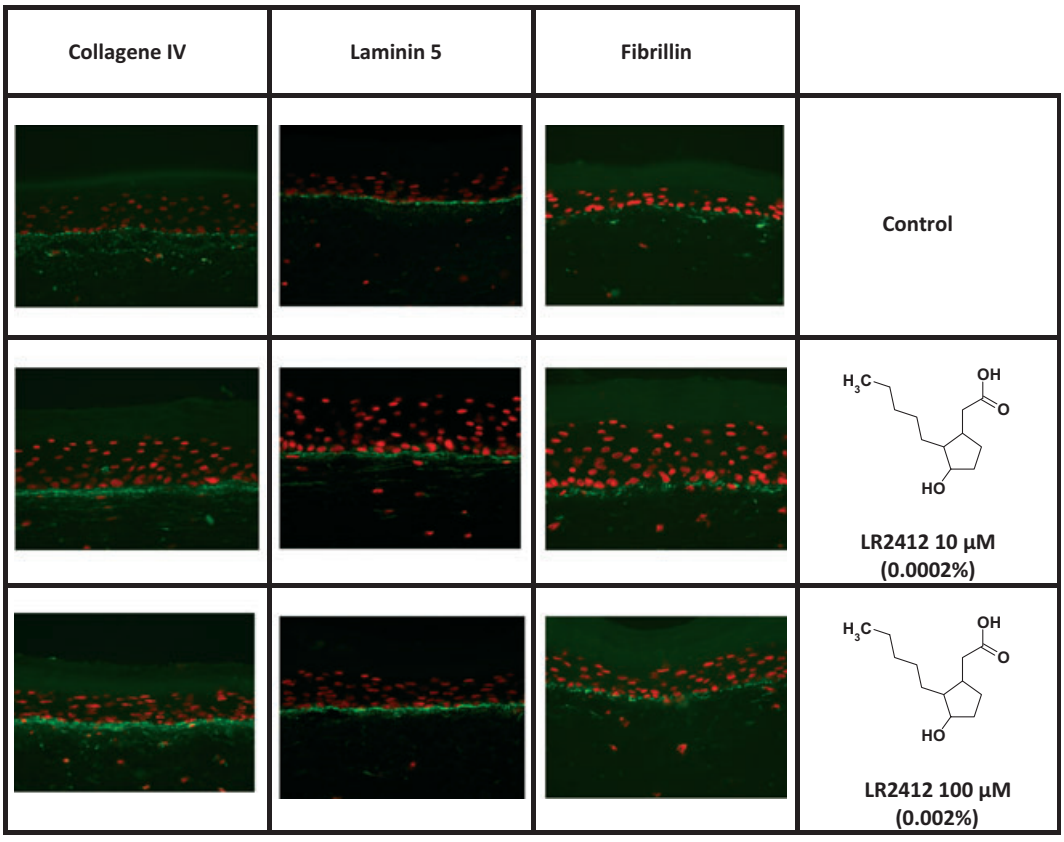

Figure 3 Immunohistochemistry of extracellular matrix proteins in REALSKIN®. Representative immunohistological sections of REALSKIN® (reconstructed human skin) after five days exposure to 10 μmol/L LR2412 reveal compared with non-treated controls, an increased deposition of collagen IV and laminin-5 at the DEJ and fibrillin in the upper dermis.

level of the DEJ as well as the deposition of fibrillin in the upper dermis as revealed by immunohistochemistry (Fig. 3).

Reversal of steroid-induced skin atrophy

Figure 4a shows that at day 1 (D1) all treated groups do not present signs of atrophy. Atrophy in the different groups varies significantly with time ($P < 0.0001$), increasing from D28 to D56 and decreasing from D56 to D67 significantly ($P = 0.0012$). At D56, LR2412 treated areas show a significantly reduced atrophy compared with placebo treated and untreated areas ($P = 0.0274$ and 0.0009, respectively). Histological evaluation (Fig. 6b) shows that at D56, areas treated with LR2412 present a significantly thicker epidermis compared with untreated areas ($P = 0.0190$) as measured by histometry.

Patch test study

As previously described, *t*RA (the clinical 'gold' standard) produced a significant deposit of fibrillin-rich microfibrills in the papillary dermis compared with that observed at baseline ($P = 0.019$). Application of vehicle, following the 12-day patch-test assay, produced little effect on fibrillin-rich microfibril deposit ($P > 0.05$). In contrast, application of 2% LR2412 resulted in a significant deposit of fibrillin-rich microfibrils (Fig. 5), the accumulation being at a similar level to that observed using either retinol or *t*RA (mean ± SE; baseline, 2.00 ± 0.27; vehicle formulation, 2.28 ± 0.31; test product, 2.79 ± 0.26*; retinol, 2.89 ± 0.23**; *t*RA, 2.85 ± 0.23**; $*P < 0.05$, $**P < 0.01$). As in previous studies, treatment with *t*RA had little effect on pro-collagen I or collagen VII deposit.[16]

3-month facial application

Applying the « Shadow method » on Silflo® imprints (Fig. 6), we observed a slight reduction of wrinkles for the vehicle. In subjects treated with LR2412 compared with vehicle, the total wrinkle surface decreased at D28 and D84 ($P < 0.05$). We also observed a significant difference at D28 and D84 ($P < 0.05$) for the total wrinkle surface, and a trend for wrinkle length ($P < 0.06$ at D84 for wrinkle length).

These data demonstrate the efficacy of LR2412 to reduce crow's feet wrinkles after 3 months application.

Discussion

Based on the promising results of LR2412 during the preliminary evaluation studies in terms of its safety profile and biological activity, in-depth studies were performed to further evaluate its potential as a skin 'anti-ageing' agent. As the ageing process

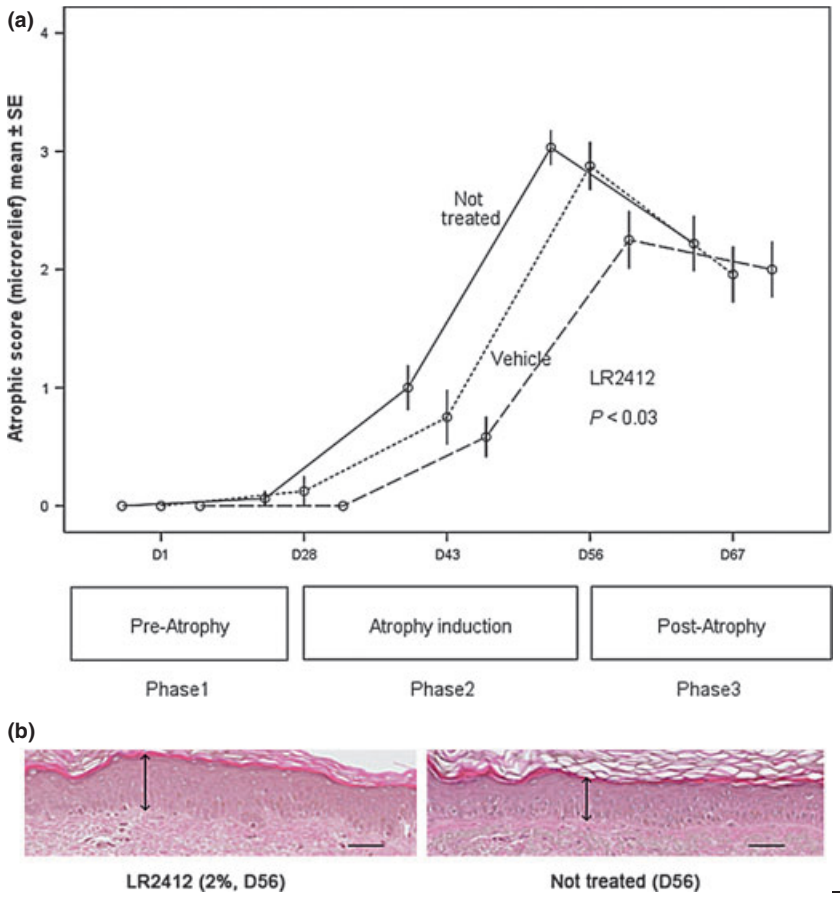

Figure 4 Reversal of corticoid induced atrophy by LR2412. (a) At baseline (D0), all treated groups are comparable, presenting no signs of atrophy. The degree of atrophy varied significantly with time ($P < 0.0001$), increasing from D28 to D56 and significantly decreasing from D56 to D67 ($P = 0.0012$) between the different treatment groups. At D56, LR2412-treated areas present a significantly reduced atrophy score as compared with placebo and non-treated areas ($P = 0.0274$ and 0.0009, respectively). (b) Histological evaluation shows that at D56, areas treated with LR2412 present a significantly thicker epidermis compared with untreated areas ($P = 0.0190$).

affects all skin layers, penetration studies were performed to assure that LR2412 passes the *stratum corneum* barrier and reaches the underlying living structures including the dermis (Table 1). The results of X-ray diffraction studies (Fig. 1) revealed integration of LR2412 into the intercellular lipids of the *stratum corneum* and excellent penetration into the underlying layers, probably due to its amphiphilic physico-chemical characteristics, allowing LR2412 to reach the superficial structures of the dermal compartment.

Corneocyte desquamation at the skin surface is an important physiological process to maintain epidermal homeostasis. Corneocytes adhere to each other via corneodesmosomes, in particular via the glycoprotein corneodesmosin, which is progressively proteolysed by serine proteases during desquamation.[18] Dry, xerotic and 'winter' skin, are very common disorders in the aged, and xerosis is characterized by an impaired proteolytic degradation of corneodesmosomes leading to thickened stratum corneum with a rough surface[19] and LR2412 induced desquamation in isolated human stratum corneum. As observed in subsequent electron microscopy studies, LR2412 induced desquamation due to a loss of corneocyte cohesion at the level of corneodesmosomes in the upper layers of the stratum corneum, as described *in vivo* (Fig. 2). The results observed could be, at least partially, explained by physicochemical properties of LR2412. Indeed, one can predict that the surfactant properties of LR2412 are due to the presence of an acidic function and an alkyl side chain on a cyclopentane core. However, it is probable that the whole structure of the molecule contributes to its desquamation properties since a surfactant property is not a guarantee for effectiveness in this process. In addition, confirming the perfect fit of the LR2412 as a smooth desquamation agent in cosmetic applications, this jasmonate derivative is very well tolerated unlike efficient but irritating desquamation molecules, such as the ionic surfactant sodium lauryl sulfate.

The DEJ separates the epidermis from dermis. Composed of ECM proteins that include collagen IV, laminin-5 and fibronectin,[20] it has not only mechanical properties, but is also implicated in controlling the diffusion and exchange of molecules between the dermis and epidermis, including those that may influence keratinocyte proliferation.[21] Ageing of the DEJ, due to

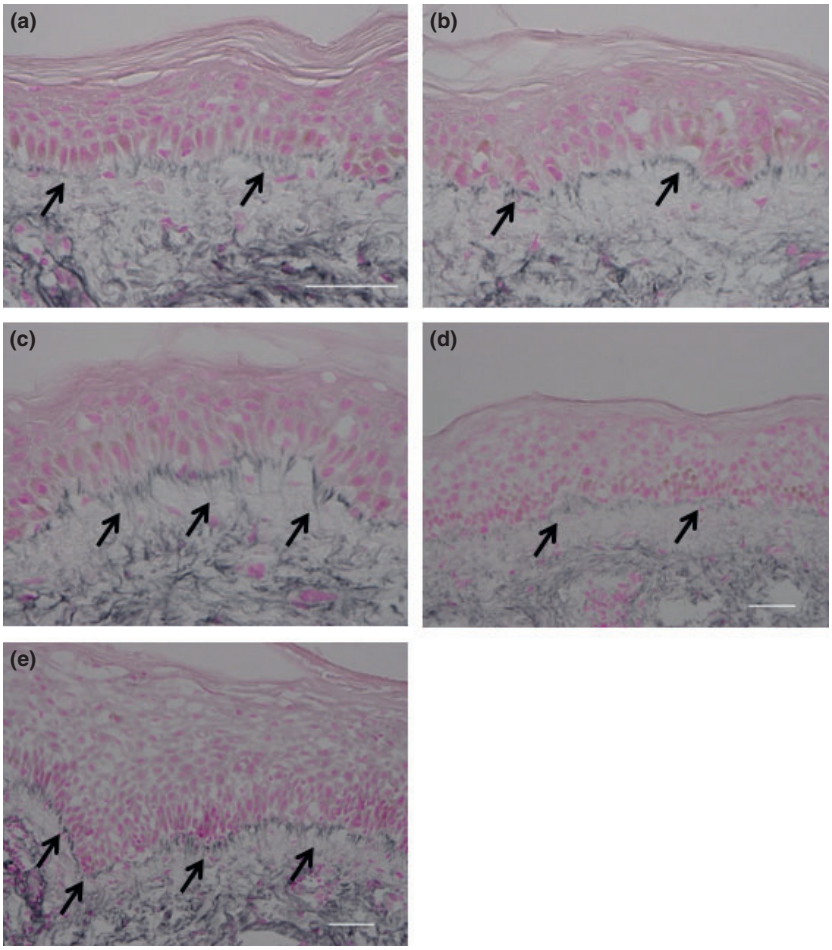

Figure 5 Application of LR2412 results in deposition of fibrillin-rich microfibrils in the papillary dermis of chronically photoaged skin. Sections of treated skin were assessed for the presence of fibrillin-rich microfibrils by immunohistochemistry. Arrows indicate the presence of these microfibrils in: (a) untreated, photoaged skin and following treatment with: (b) vehicle; (c) 2% LR2412; (d) 0.07% retinol and (e) 0.025% all-*trans* RA. Scale bar 50 μm. (f) Quantification of immunostaining, $*P < 0.05$ as compared with baseline untreated skin.

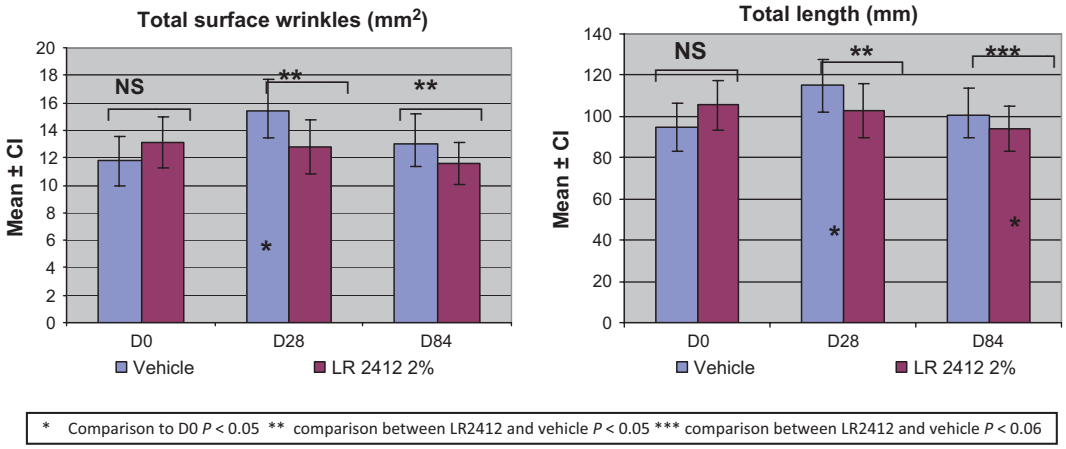

Figure 6 Effect of LR2412 (2%) on crow's feet wrinkles at D0, after 1 month (D28) and 3 months (D84) application measured by Image analysis of Silflo replicas. Applying the « Shadow method » on Silflo imprints), we observed a slight reduction of wrinkles for the vehicle. Total wrinkle surface and length after 1-month treatment with LR 2412 showed an improvement ($P < 0.05$) that became statistically significant at D84 ($P < 0.05$) compared with D0 for the wrinkle length. Compared with the vehicle, this difference was significant at D28 and D84 ($P < 0.05$) for the total wrinkle surface as well as for wrinkle length ($P < 0.06$ at D84 for wrinkle length).

alterations in its components and mainly a reduction of collagen IV[1] causes a strong reduction of its surface area and the loss of dermal papillae.[22] We show, using a skin equivalent model (REALSKIN®), that LR2412 is able to stimulate deposition of laminin-5, collagen IV and fibrillin close to the DEJ. Based on these *in vitro* results, we performed clinical trials to confirm *in vivo* the activity of LR2412.

First, a proof of principle study utilizing a model of skin ageing was performed; for many years, steroid-induced effects are proposed as a functional and dynamic model of skin ageing.[23] Indeed, systemic[24] as well as topical application of corticosteroids[25] induces skin atrophy. Analysis of the steroid-induced alterations reveal that they are very similar to those observed in atrophic, aged epidermis[26] with reduced keratinocyte proliferation and glycosaminoglycan synthesis.[25,27] It has been shown that steroid-induced epidermal atrophy can be reduced by topical application of all-*trans* retinoic acid.[24] We have applied a similar protocol to evaluate the potential of LR2412 to reverse epidermal atrophy. As shown in Fig. 4a,b, LR2412 has significant efficacy to reverses corticoid-induced skin atrophy**.** These results demonstrate that topical LR2412 reverses clinical signs of skin ageing and in an extended manner dermatoporosis.[28]

Secondly, in a validated double-blind patch test assay, LR2412 was applied to photoaged skin and compared with retinol and all-*trans* retinoic acid; LR2412 significantly increased the deposition of fibrillin-rich microfibrils in the papillary dermis proximal to the DEJ, the accumulation being at a similar level to that observed using retinol or *t*RA (Fig. 4). These results confirm our *in vitro* observation of fibrillin deposition in the upper dermis of REALSKIN®.

Finally, in an in use clinical study, performed in 42 women over 3 months, we showed that applying a product containing 2% LR2412 twice a day significantly reduced the area and length of crow's feet wrinkles on the face (Fig. 5). This might be explained by the fact that LR2412 induced *in vitro* and *in vivo* the deposition of fibrillin rich-microfibrils the degradation of which during the process of photoageing may contribute to the loss of skin elasticity and wrinkle formation.[29] It is important to note that during our clinical studies we did not observe notable adverse effects of LR2412 such as inflammation or irritation.

In conclusion, results from both *in vitro* and *in vivo* studies show that LR2412 has the capacity to target different compartment of the skin affected by intrinsic and extrinsic ageing processes, and therefore has potential to partially correct signs of skin ageing.

Acknowledgements

The authors thank J. Doucet from Phi Axis (Anthony, France) for the analysis of X-ray data, D. Bernard (L'Oréal Research and Innovation) for the desquamation assay, M. Dalko (L'Oréal Research and Innovation) for the chemical synthesis of LR2412, Dr C. Queille-Roussel (CPCAD, Nice,), R. Bazin and J. Laboureau (L'Oréal Research and Innovation) for clinical studies and Dr M. Verschoore for manuscript revision.

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