





Report

UVA-1 phototherapy as adjuvant treatment for eosinophilic fasciitis: *in vitro* and *in vivo* functional characterization

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Conflict of interest: None.

Funding source: None. Open Access

Funding provided by Università degli Studi di Siena within the CRUI-CARE Agreement.

doi: 10.1111/ijd.16003

Introduction

Eosinophilic fasciitis (EF) is a rare autoimmune disease that causes a progressive induration of dermal, hypodermal, and muscularis fascia of the trunk and extremities.^{1–4} The exact pathogenesis is yet to be fully understood: it is supposed that a certain trigger—trauma, drug, infection—stimulates an inflammatory local fibrotic reaction involving external muscularis fascia and overlying tissues.^{4–6}

A validated specific protocol for treatment and management of EF is still lacking. Patients are usually managed with the immunosuppressive combination of prednisone and methotrexate, even for a long time, until remission.^{7,8} UVA-1 phototherapy was employed in many sclerosing diseases of the skin in the last decades, but there are few recent reports as an adjuvant treatment

Abstract

Introduction Eosinophilic fasciitis (EF) is a rare autoimmune disease causing progressive induration of dermal, hypodermal, and muscularis fascia. The exact pathogenesis is yet to be fully understood, and a validated therapy protocol still lacks. We here aimed to realize a clinical–functional characterization of these patients.

Materials and methods A total of eight patients (five males, 45 years average) were treated with adjuvant high-dose UVA-1 phototherapy (90 J/cm), after having received the standard systemic immunosuppressive protocol (oral methylprednisolone switched to methotrexate). Body lesion mapping, Localized Scleroderma Assessment Tool (LoSCAT), Dermatology Life Quality Index (DLQI), High-Resolution Ultrasound (HRUS) (13–17MHz), and ultra HRUS (55–70 MHz) were performed at each examination time taking specific anatomical points. Gene expression analysis at a molecular level and *in vitro* UVA-1 irradiation was realized on lesional fibroblasts primary cultures.

Results The LoSCAT and the DLQI showed to decrease significantly starting from the last UVA-1 session. A significant reduction in muscularis fascia thickness (–50% on average) was estimated starting from 3 months after the last UVA-1 session and maintained up to 12 months follow-up. Tissues was detected by HRUS. The UVA-1 *in vitro* irradiation of lesional skin sites cells appeared not to affect their viability. Molecular genes analysis revealed a significant reduction of *IL-1β* and of *TGF-β* genes after phototherapy, while MMPs 1,2,9 gene expression was enhanced.

Comment These preliminary *in vivo* and *in vitro* findings suggest that UVA-1 phototherapy is a safe and useful adjuvant therapy able to elicit anti-inflammatory effects and stimulate tissue matrix digestion and remodeling at lesional sites.

also in EF.^{9,10} As per disease monitoring, there are some experiences with medium resolution ultrasound (US) only.^{8,11}

We first aimed to obtain a thorough clinical–functional characterization of EF patients undergoing an adjuvant UVA therapy, using high-resolution US, second, to evaluate the response to UVA-1 irradiation *in vitro* of human fibroblast primary cultures from lesional skin, and third, to investigate the gene expression profiling of both profibrotic and antifibrotic pathways before and after *in vivo* UVA-1 irradiation.

Materials and methods

The study was carried out in the Dermatology Unit, Skin Bank and Skin Cultures Laboratory of Siena University Hospital (Italy), in collaboration with the Department of Dermatology and

Pathology of Saint Etienne (France), realized in accordance with the Declaration of Helsinki and approved by the local ethical committee. Informed consent was obtained, and all data were de-identified before use.

Study protocol

A total of eight patients aged between 30 and 58 years with clinical and laboratory suspicion of EF were enrolled for this study within September 2016 and September 2019. Inclusion criteria were age >18 years, eligibility for standard therapy, that is, oral prednisone (OMP) + intramuscular methotrexate (MTX), eligibility for UVA-1 adjuvant whole-body phototherapy. After skin biopsy, standard therapy was started as follows: Phase I: OMP tapered from 3 mg/kg/d for 3 months, Phase II: MTX 15 mg/week + OMP 8–16 mg/d for 3 months, Phase III: maintenance MTX once a week for 3–6 months, and Phase IV: ending according to clinical response (within 6 months). Adjuvant phototherapy was performed starting from Phase III of systemic therapy. Timeline for examinations thus included as follows: baseline/first irradiation/skin biopsy (t_0), last irradiation session (t_1), 2 weeks after t_1 (t_2), 1 month after t_1 (t_3), 2 months after t_1 (t_4), 3 months after t_1 (t_5), 6 months after t_1 (t_6), and 9 months after t_1 (t_7).

Clinical monitoring

Localized Scleroderma Assessment Tool (LoSCAT),^{12–14} normal modified Rodnan Skin Score (mRSS), and Dermatology Life Quality Index (DLQI) scores were calculated at each examination time t_0 – t_7 . A body mapping of EF lesions that were clinically visible and detectable with palpation was realized according to a dedicated scheme at t_0 , t_5 , t_6 , and t_7 (Fig. 1a).

Ultrasound monitoring

High-frequency Ultrasound (HFUS) and ultra-high-Frequency Ultrasound (uHFUS) were realized with linear probes 22 MHz and 13–17 MHz (*MyLab™ Twice Esaote biomedical*) and 55–70 MHz linear probe (*VEVO MD®*, VisualSonics Fujifilm),

respectively. HFUS and uHFUS were performed at t_0 , t_3 , t_5 , t_6 , and t_7 to assess the thickness of dermal, hypodermal, and fascial tissues at a lesional point and in contralateral healthy points, namely: dermis, hypodermis, external muscularis fascia. In order to standardize HFUS measurements, five lesional sites were examined on the abdomen, arm, forearm, thigh, and leg, localized at specific anatomic *reper*e points (Fig. 1b).

UVA-1 adjuvant therapy

High-dose-regimen UVA-1 irradiation (*GP24H medical bed*, Cosmedico®, Medizintechnik) was administered as whole-body irradiation of 90 J/cm for 40 consecutive sessions, 3–4 times/week.^{2,7} A pretreatment with two low doses (30 J/cm²) and three with medium dose (70 J/cm²) administrations ensured adequate skin preparation in phototype III patients. Patients with phototypes I–II were tested for minimal erythema dose¹⁵ and were pretreated accordingly (i.e., sessions of 20, 40, and 60 J/cm²). Patients were recommended to wear a pair of protective tanning eyewear during all sessions.

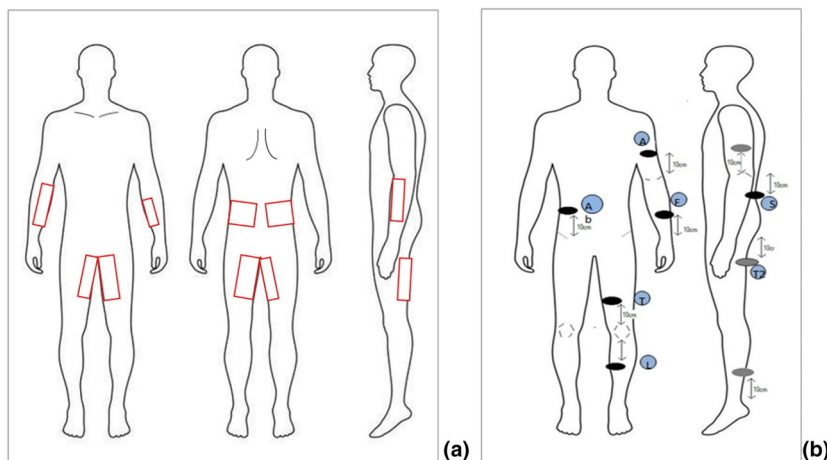
Histologic examination

Lesional specimens were harvested at t_0 and t_5 for histopathologic examination and primary cultures set up at two lesional sites, thigh (eight patients) and forearm (three arms). Conventional incisional biopsy dissecting the dermis until reaching the external muscularis fascia of the femoral quadriceps was performed on the thighs. For the arm site, a microinvasive bioptic technique was adopted, using an 18G × 250 mm needle combined with Pistolet Bard Magnum, conventionally used for the prostatic biopsy, having an inner diameter of <2 mm, thus generating a minimal scar on the lesional tissue.

Primary cell cultures

Primary cultures were obtained from lesional bioptic before (t_0) and after (t_5) UVA-1 adjuvant therapy. Primary cultures (P0)

Figure 1 Clinical mapping was performed before, during, and after UVA-1 adjuvant phototherapy for cutaneous signs of eosinophilic fasciitis (a) and ultrasound mapping (b) for dermal, hypodermal, and fascial examination at established *reper*e points



were expanded until the fourth passage (P4), incubated with DMEM culture medium supplemented with 10% fetal bovine serum, 5 mM penicillin/streptomycin, 2 mM L-glutamine (Euroclone, Devon, UK) in a 5% CO₂ incubator at 37°C control fibroblasts, that served for *in vitro* irradiation and molecular analyses, were obtained from healthy tissue harvested from patients undergoing dermatologic surgery, matched for body sites, age, and sex. P4 cells were differently processed according to subsequent experiments, which were repeated at t_0 and t_5 for both lesional and healthy populations.

In vitro UVA-1 irradiation

Four groups of fibroblasts were assigned to different irradiation doses, namely: 0.1, 0.5, 1, and 5 J/cm², corresponding to increasing irradiation time of 42 seconds, 3 minutes 30 seconds, 7 minutes, and 15 minutes. Cells were seeded into 5 mm Petri dishes with DMEM as previously described; to avoid any possible interference with the phenolic pigment of DMEM, cells were washed and seeded with 1.5 µl phosphate buffer saline (PBS) right before irradiation.¹⁶ This was administered with a Solar Simulator paired with a 150 W Xenon Lamp (Thermo-orient, Oriol Instruments®) when cells were semiconfluent (~650,000 cells/Petri dish). The device was customized with an air mass filter lens and a bandpass lens (335–610 nm) to emit only UVA-1 rays in the spectral region of 340–400 nm. The distance between the cell monolayer and the lens was standardized to 1.5 cm.

Cell viability monitoring

MTT metabolic assay (tetrazolium salts [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) was performed in order to evaluate the viability at 0, 24, 48, and 72 hours after *in vitro* irradiation; results were quantified spectrophotometrically measuring the optical density (OD) at 570 nm (Thermo Scientific Evolution 60S) as previously described.^{17,18}

Morphologic monitoring, cell counting, and measurements were performed for each cell group every 7 days through a binocular invertoscope (Nikon ECLIPSE Ts2) able to take sequential imaging, in combination with a Neubauer glass counting chamber for field counting (Fig. 6).

Molecular analysis

Adherent cells were collected with 1 ml of Trifast (Ambion, Austin, TX, USA) for RNA concentration assessment (Nanodrop Spectrometer, Thermo Fisher Scientific, Delaware); RNA integrity was evaluated by an electrophoretic run on agarose gel with FlashGel System (Lonza Group, Switzerland). A total of 300 ng of extracted RNA was reverse transcribed into cDNA using the iScript™ cDNA Synthesis kit (Bio-Rad Laboratories, USA). Specific primers for interleukin-1β (IL-1β), transforming growth factor β (TGF-β), metalloproteinase 1, 2, and 9 (MMP-1, MMP-2, and MMP-9), and β-actin were designed for the present study by using the software Primer_BLAST (available

at: <https://blast.ncbi.nlm.nih.gov>). Expression levels of these genes were determined by qRT-PCR on a CFX Connect using 1X SSOFAST EvaGreen Supermixes (Bio-Rad Laboratories). The selected reference gene encoding for β-actin was used to normalize Ct values, and quantities were calculated in relation to the maximum Ct value.

Zymography analysis

Gelatin zymography was performed at t_0 and t_5 on both healthy and lesional tissue on the gelatinases MMP-2 and MMP-9.¹⁹ Supernatants protein content was evaluated with the Bradford method²⁰; 7.5% sodium dodecyl sulfate (SDS)–polyacrylamide gel (PAG) was copolymerized with 0.1% gelatin, and 20 mg of protein of each sample was loaded into each well under nondenaturing conditions and run under a constant current (25 mA). After electrophoresis, the gel was rinsed in 2.5% Triton X-100 and 50 mmol/l Tris–HCl (pH 7.5) and then incubated overnight in an activation buffer (50 mmol/l Tris, pH 7.5, 0.15 mol/l NaCl, 10 mmol/l CaCl₂, and 1% Triton X-100). The gel was stained with 0.25% Coomassie Brilliant Blue, 50% methanol, and 10% acetic acid and destained with 30% methanol and 10% acetic acid.

Statistical analysis

Descriptive statistics, including frequency count, the mean and standard deviation for quantitative variables, frequency count, and percentage for qualitative variables, were computed. The Kolmogorov–Smirnov test was applied to check all quantitative variables. The *t* test and one-way analysis of variance (ANOVA) were used to compare groups when distributions were normal, then Bonferroni post hoc test for pairwise comparisons. A *P* value of <0.05 was considered significant.

Results

Case study and UVA-1 phototherapy

Enrolled patients were aged 45 years on average, five males and three females, of phototype II (four cases), III (three cases), and I (one case) and average body mass index of 23.5 ± 20.1–25.7. Clinical history was positive for repetitive mild traumatism due to work or sport (three cases), parasitic gastrointestinal infection (two cases), and for professional exposure to chemical vapors (five cases). Complete laboratory screening for systemic scleroderma (SSc) and rheumatoid arthritis was negative in all cases. Concomitant conditions included atopic dermatitis (two cases), lichen ruber planus (one case), type I diabetes (two cases), and osteoporosis (three cases). The time interval between t_0 (i.e., first UVA-1 session) and lesion onset was 6 months on average (range 2.8–11.6 months), whereas the time interval between t_0 and systemic therapy start was 3.5 months on average (range 0.2–6 months). Monthly follow-up control was set up.

Clinical monitoring

Clinical and ultrasound mapping were updated during t_0 – t_7 visits (Fig. 1) as well as LoSCAT, mRSSS, and DLQI scores were assessed from t_0 to t_7 (Fig. 5b,c). A significant reduction in the consistency of lesional tissue, at palpation, was found along with a progressive increase in skin elasticity and joint mobility, starting from t_4 (Figs. 3 and 4). Increased pigmentation due to the melanin stimulation of the UVA-1 whole-body phototherapy was taken into account when assessing the LoSCAT index and differentiated from dispigmentary outcomes due to EF. LoSCAT and DLQI showed to decrease significantly after t_1 and t_3 , respectively. The mRSSS score decreased from 24 at t_0 to 5 at t_7 , on average.

Ultrasound examination

Considering the forearm (F) *reper* point, the following thickness was estimated, on average, for the dermis: 1.2 ± 0.7 mm at t_0 , 0.9 ± 0.3 mm at t_5 , 0.8 ± 0.2 mm at t_7 ; for the hypodermis: 0.5 ± 0.25 mm at t_0 , 0.4 ± 0.15 mm at t_5 , 0.4 ± 0.15 mm at t_7 ; for the external muscular fascia: 1.2 ± 0.2 mm at t_0 , 0.8 ± 0.3 mm at t_5 , 0.5 ± 0.1 mm at t_7 . Considering the thigh (T) *reper* point, the following thickness was estimated, on average, for the dermis: 1.9 ± 0.3 mm at t_0 , 1.3 ± 0.25 mm at t_5 , 1 ± 0.2 mm at t_7 ; for the hypodermis: 0.9 ± 0.3 mm at t_0 , 0.9 ± 0.2 mm at t_5 , 0.8 ± 0.25 mm at t_7 ; for the external muscular fascia: 1.4 ± 0.3 mm at t_0 , 0.9 ± 0.25 mm at t_5 , 0.6 ± 0.1 mm at t_7 . Considering the leg (L) *reper* point, the following thickness was estimated, on average, for the dermis:

0.5 ± 0.25 mm at t_0 , 0.5 ± 0.3 mm at t_5 , 0.4 ± 0.35 mm at t_7 ; for the hypodermis: 0.6 ± 0.15 mm at t_0 , 0.5 ± 0.1 mm at t_5 , 0.5 ± 0.2 mm at t_7 ; for the external muscular fascia: 1.3 ± 0.2 mm at t_0 , 1.1 ± 0.4 mm at t_5 , 0.8 ± 0.15 mm at t_7 . Taking into account, all measurements were collected at four *reper* points in 11 patients, the thickness reduction of the external muscular fascia was reduced by -40% from t_0 to t_5 , -50% from t_0 to t_6 , and -60% from t_0 to t_7 . The dermal thickness was also reduced -30% from t_0 to t_5 , -35% from t_0 to t_6 , -40% from t_0 to t_7 (Figs. 2–4). In Figure 5a, the average values of tissue HFUS measurements obtained from the measurements of the dermis and of the external muscular fascia are reported along with the average LoSCAT and DLQI progress, showing a significant reduction starting from t_5 (i.e., 3 months after the last UVA-1 phototherapy session) and maintained at t_7 (i.e., 9 months after the last UVA-1 phototherapy session).

Cell culture imaging

Healthy control human fibroblasts in P1, day 14 of culture, had a cell density of $\sim 1,000,000$ cells/flask 25 cm^2 on average, 45 ± 10 nm short transversal diameter, and 500 ± 50 nm longitudinal diameter. Human fibroblasts obtained from lesional dermis before and after whole-body UVA-1 phototherapy were normal in morphology. Baseline lesional fibroblasts in P1, day 14, had a cell density of $\sim 1,200,000$ cells/flask 25 cm^2 on average, 55 ± 20 nm short transversal diameter, and 600 ± 60 nm longitudinal diameter. Post-phototherapy lesional fibroblasts harvested at t_5 , in P1, day 14, had a cell density of $>1,000,000$

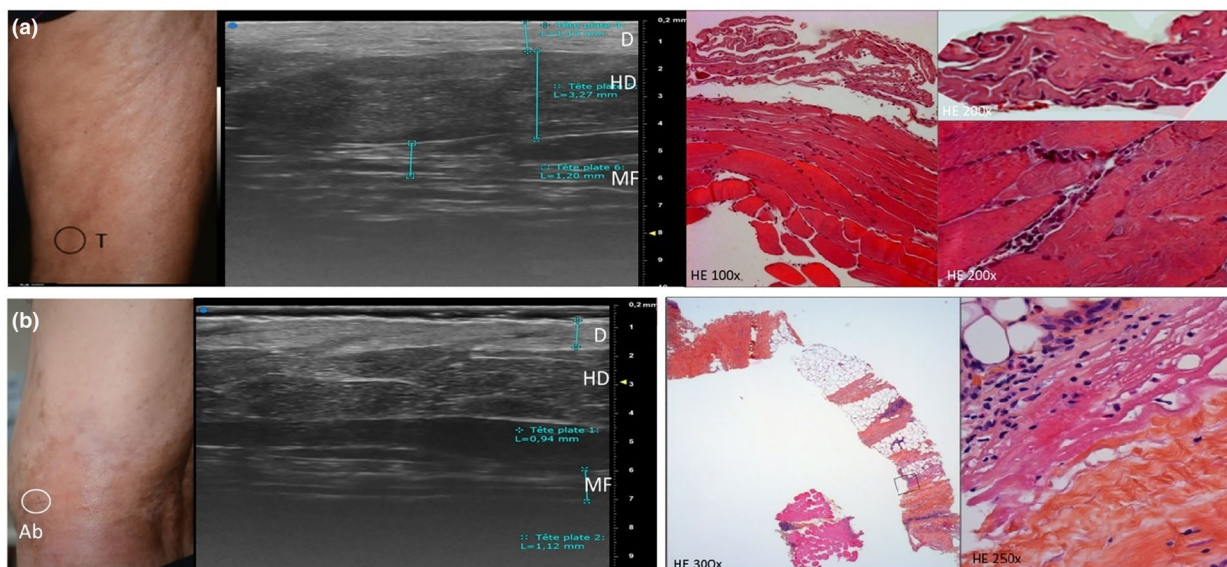


Figure 2 Baseline ultra-high-resolution ultrasound (uHRUS) of two reper points of the tight-T (a) and abdomen-Ab (b), in a 35-year-old patient with eosinophilic fasciitis: the muscularis fascia (MF), usually not visible as a linear acoustic interface, is detectable as multiple parallel echoes ranging from 1 to 1.5 mm, whereas the dermal (D) and hypodermal (HD) layers appear hyperechogenic due to the tissue-reactive fibrosis. Hematoxylin and eosin staining of traditional incisional biopsy (a) reaching the muscularis fascia and muscular tissue dermal, where eosinophils are visible at higher magnification; microbiopsy obtained with an 18G (<2 mm inner diameter) needle (b)

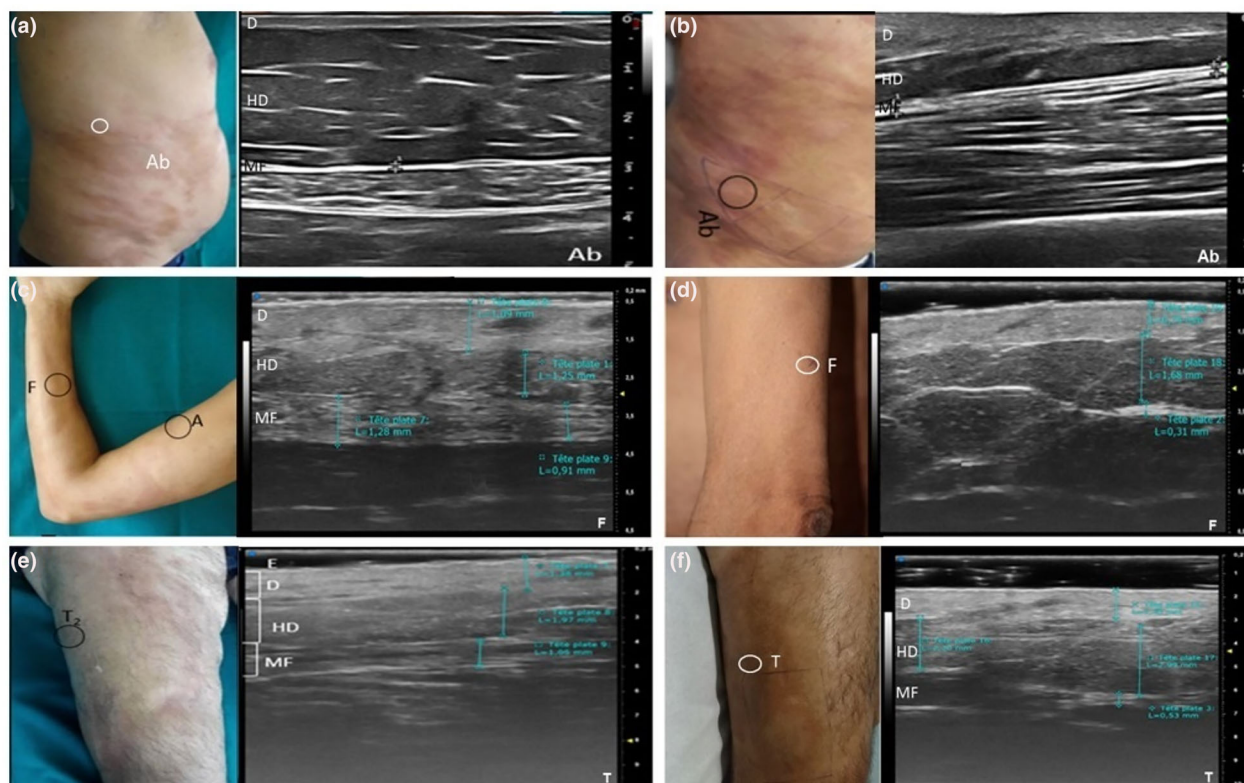


Figure 3 Ultrasound mapping realized by 50 MHz and 15 MHz of three reperi points (abdomen, Ab; forearm, F; and thigh, T) was performed in a 45-year-old patient with eosinophilic fasciitis at baseline (a) and 3 months post-UVA-1 adjuvant phototherapy (t_3) (b): the thickness of the muscularis fascia (MF) is significantly reduced at t_3 in all reperi points, whereas the dermis (D) and hypodermis (HD) is morphologically unaltered

cells/flask 25 cm^2 on average, $40 \pm 15 \text{ nm}$ short transversal diameter, and $500 \pm 60 \text{ nm}$ longitudinal diameter. Human lesional fibroblasts harvested at baseline and irradiated *in vitro* with crescent UVA-1 doses did not show significant alteration in morphology, growth and average length at 0, 24, 48, and 72 hours after irradiation (Fig. 6a). After 24, 48, and 72 hours, the longitudinal diameters were measured 550, 500, and 480 nm on average, whereas the transversal diameters were measured 60, 55, and 50 nm, respectively.

In vitro UVA-1 irradiation

The average value of cell viability obtained by MTT metabolic assay from all analyzed specimens is reported in Fig. 6b. Globally, a decay in the $\text{OD}_{570\text{nm}}$ of $\sim 30\%$ from 0 to 72 h after irradiation was observed. The average value for the fibroblasts irradiated with 0.1 mJ/cm^2 UVA-1 dose was 0.111 ± 0.01 at 0 hours, 0.10 ± 0.02 at 24 hours, 0.091 ± 0.03 at 48 hours, and 0.083 ± 0.02 at 72 hours. The average value for the fibroblasts irradiated with 0.5 mJ/cm^2 UVA-1 dose was 0.110 ± 0.03 at 0 hours, 0.10 ± 0.02 at 24 hours, 0.099 ± 0.03 at 48 hours, and 0.081 ± 0.02 at 72 hours. The average value for the fibroblasts irradiated with 1 mJ/cm^2 UVA-1 dose was 0.113 ± 0.03 at 0 hours, 0.097 ± 0.03 at 24 hours, 0.085 ± 0.05 at 48 hours,

and 0.079 ± 0.4 at 72 hours. The average value for the fibroblasts irradiated with 5 mJ/cm^2 UVA-1 dose was 0.112 ± 0.03 at 0 hours, 0.092 ± 0.02 at 24 hours, 0.083 ± 0.03 at 48 hours, and 0.075 ± 0.02 at 72 hours.

Molecular analysis

At baseline, lesional tissue exhibited an overexpression of IL-1 β compared with healthy controls, normalized after phototherapy (Fig. 6c); the same results were observed for TFG- β . On the contrary, the MMP-1, MMP-2, and MMP-9 increased significantly after phototherapy; zymographic data confirmed the stronger intensity of all MMPs genes in treated lesional cells compared with baseline lesional and healthy cells (Fig. S1).

Comment

EF is a rare condition with about 350 cases reported to date: its understanding relies essentially on case series and case reports and continues to evolve. Some authors considered EF as a deep form of localized scleroderma (LS): nevertheless, 28–65% of EF patients develop LS lesions.^{7,8,21,22} The mRSS has been traditionally used to monitor the disease activity in SSc and LS patients and occasionally for EF patients.^{7–11,23–25} However, the

mRSS has multiple limitations, including the impossibility to identify slight alterations in skin thickness and to take into account high intraobserver/interobserver variability. We thus decided to perform both mRSS and LoSCAT indices at each examination: the LoSCAT appeared to be sensitive to patient improvements, whereas the mRSS did not exhibit a clear correlation.²² Nevertheless, attention should be paid by physicians in distinguishing postinflammatory pigmentation from UVA-1-induced darkening in patients with III–IV skin phototypes.⁹ It has been postulated that the antifibrotic UV-induced effects reach a plateau after an intense level of skin pigmentation and that besides that point the consecutive irradiation has no effect (i.e., no significant decrease of procollagen).^{26,27} We indeed observed that patients with skin phototypes I and II achieved a

more rapid and intense skin elasticity compared with phototype III patients.

The medium-resolution US (7–15MHz) has been employed to monitor disease activity in EF patients,^{24,28,29} but no specific and globally accepted protocol for lesional skin examination exists.¹¹ To the best of our knowledge, examinations with HRUS (22 MHz) and uHRUS (50 MHz) were never reported in EF patients. This new protocol allowed us to obtain a thorough measurement of the fascial, dermal, and hypodermal thickness and to simultaneously detect pathognomonic morphologic details of the external muscularis fascia (e.g., lack of compressibility, increased thickness, hyperechoic tram-track appearance) (Figs. 2, 3) along with signs of inflammation of the hypodermis and dermis (e.g., tissue density, fibrosis, vascular flow).^{30–33}

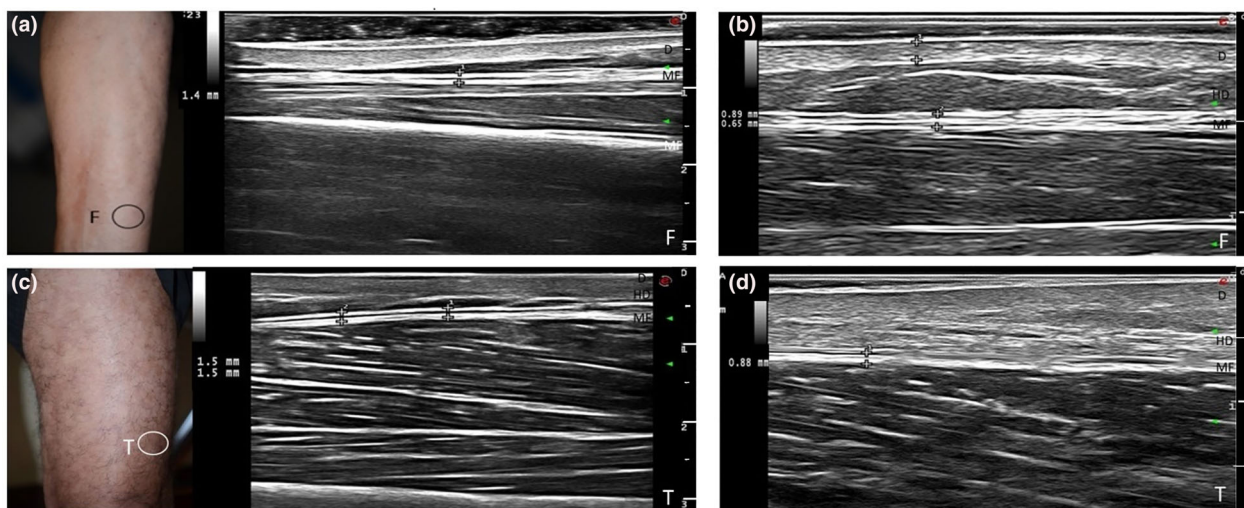


Figure 4 Ultrasound mapping realized by 15 MHz of two repere points (forearm, F and thigh, T) performed in a 40-year-old patient at baseline (a, c) and 3 months post-UVA-1 adjuvant phototherapy (t_5) (b, d): the thickness of the muscularis fascia is significantly reduced at both F and T repere point

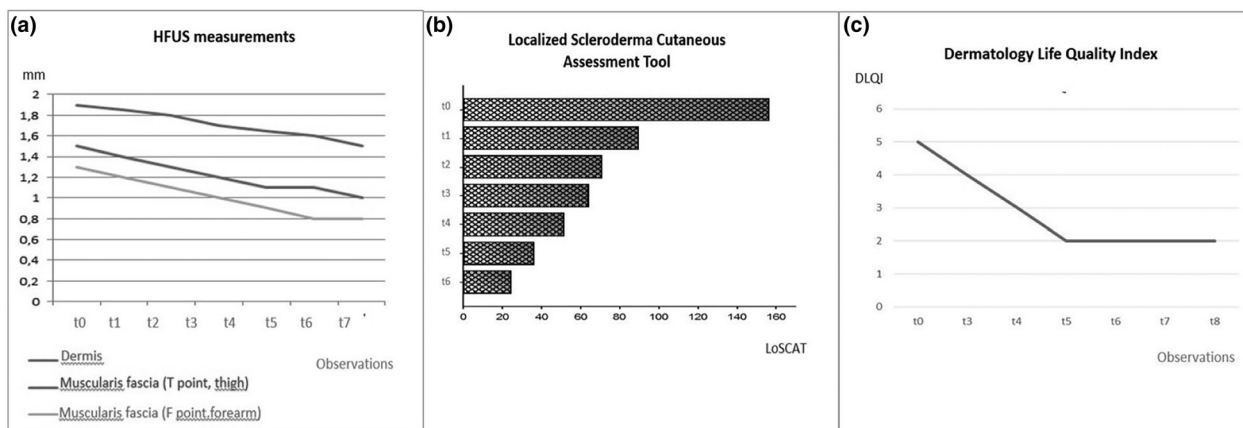


Figure 5 Summary of the t_0 – t_7 assessments of average dermal value, hypodermal, and muscularis fascia thickness by high-frequency ultrasound (a), Localized Scleroderma Cutaneous Assessment Tool (LoScat) (b), and of the Dermatology Life Quality Index (DLQI) (c)

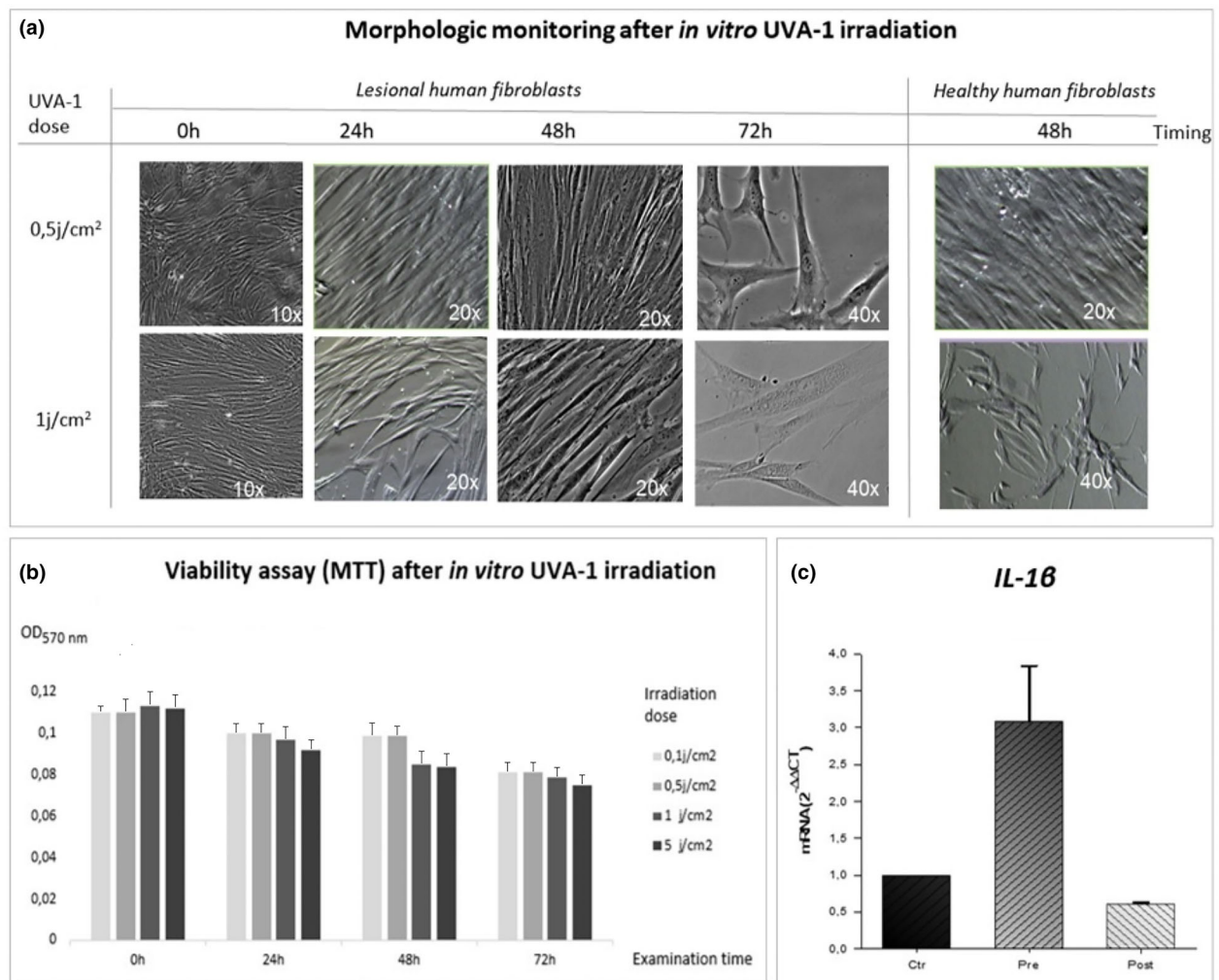


Figure 6 Human lesional fibroblasts harvested at baseline and irradiated *in vitro* with crescent UVA-1 doses, imaged at different magnifications (a), and tested for viability (MTT) (b) before and after 24, 48, and 72 hours; healthy controls are also shown (b). Normalized expression quantified by real-time PCR of interleukin-1 β (IL-1 β) and metalloproteinase 1 encoding genes, from lesional tissue specimens obtained before (pre) and after (post) 3 months UVA-1 phototherapy in patients with eosinophilic fasciitis; control patients matched for age, sex, and body site (ctr)

There is currently no universally accepted protocol for treating EF: systemic corticosteroids and immunosuppressants induce remission of disease in many cases and are effective on articular symptoms as pain and stiffness²¹ but do not impact significantly over the dermal and hypodermal rigidities.^{2,3,21} The core rationale for delivering UVA-1 phototherapy in sclerotic skin diseases, observed in *in vitro* experiments in the last decade, includes the modulation of proinflammatory cytokines, the stimulation of the interstitial matrix metalloproteinases, and the induction of apoptosis of T cells in the superficial dermis.^{26,34–37} UVA-1 phototherapy was employed in many sclerosing diseases of the skin, including LS, scleromyxedema, lichen sclerosus, lichen myxedematosus, scleredema, nephrogenic systemic fibrosis, and sclerodermic skin lesions, in patients with SSc.^{7,8} A cycle of medium-dose UVA-1 (30–59 J/cm²) or high-dose

UVA-1, 3–5 times weekly for a minimum of 30 irradiations, proved to reduce skin rigidity in LS starting from 3 to 4 months and lasting up to 3 years in half cases, otherwise a second cycle is suggested. Since it was conventionally thought that the penetration of UVA-1 rays was not deep enough to reach the muscularis fascia, UVA-1 phototherapy has been rarely delivered on deep LS subtypes with involvement of muscle and bone, having a level of recommendation for UVA-1 in EF patients is classified as 2D.³⁸ However, based on the concept that EF is a continuum with LS, UVA-1 phototherapy was recently used as adjuvant treatment in some EF cases with the aim of sparing immunosuppressant agents and improving skin and subcutaneous tissue compressibility, namely: in the form of psoralen-ultraviolet bath photochemotherapy³⁹ or as UVA-1 whole-body phototherapy plus psoralen (2 patients)²³ or plus

corticosteroid.¹⁰ Here in this study, medium-dose UVA-1 phototherapy demonstrated to be a safe and well-tolerated adjuvant treatment.

The UVA-1 *in vitro* irradiation test carried out on both lesional EF patient cells and on healthy control patient cells appeared to not impact negatively on viability and on proliferation^{27,34} (Fig. 6a). The molecular investigations here carried out, for the first time, on tissue specimens irradiated with *in vivo* UVA-1, revealed a significant reduction in key molecule IL-1 β and, in parallel, of the TGF- β gene: this generates a downstream inhibition of the collagen production pathway^{9,26} and is in line with the hypothesis of a causative role for the TGF- β released by infiltrating eosinophils in the pathogenesis of EF lesions.^{1–3,40} To date, only antifibrotic data from *in vitro* irradiation experiments on localized scleroderma cells were available.^{27,34,37} On the other hand, UVA-1 rays appeared here to induce MMPs 1, 2, and 9 genes expression, which are responsible for tissue matrix digestion and collagen remodeling. Taken together, these preliminary findings suggest that UVA-1 exerts an *in vivo* anti-inflammatory effect on deep lesional dermis in EF patients that further normalize the pro-fibrotic process involving the adjacent external muscularis fascia due to cytokine interplay.^{27,34,37}

First limitation is that the number of different primary cultures was derived from few patients; however, being a rare disease, the amount of human cell cultures derived and expanded for the experiments is considerable. The second limitation is that the molecular expression at tissue level could be, at least partially, influenced by the protocol (OMP+MTX) administered before starting phototherapy: nevertheless, the treatment with phototherapy alone is impossible for the severity of the disease itself, and the examination timing we selected allowed to observe molecular changes occurring at tissue level from 3 months after phototherapy, that coincided with the end of the MTX maintenance phase. In addition, the rapid improvement in lesional elasticity and pliability of the skin and of the dermal/hypodermal/fascial interface, and its maintainance up to 12 months, when patients are without systemic therapy, can be ascribable to UVA-1 irradiation only.

In conclusion, further studies with a higher number of EF naïve patients are needed to investigate the complex immunomodulatory effects elicited by UVA-1 rays on lesional tissue and inflammatory pathways, and to evaluate the necessity of a new clinico-functional score dedicated to EF able to combine clinical and ultrasound data.

References

- Pinal-Fernandez I, Selva-O' Callaghan A, Grau JM. Diagnosis and classification of eosinophilic fasciitis. *Autoimmun Rev* 2014; **13**(4–5): 379–382.
- Mertens JS, Seyger MMB, Thurlings RM, Radstake TRDJ, de Jong EMGJ. Morphea and eosinophilic fasciitis: an update. *Am J Clin Dermatol* 2017; **18**: 491–512.
- Wright NA, Mazori DR, Patel M, Merola JF, Femia AN, Vleugels RA. Epidemiology and treatment of eosinophilic fasciitis: an analysis of 63 patients from 3 tertiary care centers. *JAMA Dermatol* 2016; **152**: 97–99.
- Mosconi S, Streit M, Bronimann M, Braathen L. Eosinophilic fasciitis (Shulman syndrome). *Dermatology* 2002; **205**: 204–206.
- Sherber NS, Wigley FM, Paget SA. Diffuse fasciitis with eosinophilia developing after local irradiation for breast cancer. *Clin Rheumatol* 2009; **28**: 729–732.
- Kahari VM, Heino J, Niskanen L, Fraki J, Uitto J. Eosinophilic fasciitis. Increased collagen production and type I procollagen messenger RNA levels in fibroblasts cultured from involved skin. *Arch Dermatol* 1990; **126**: 613–617.
- Knobler R, Moinzadeh P, Hunzelmann N, et al. European Dermatology Forum S1-guideline on the diagnosis and treatment of sclerosing diseases of the skin, Part 1: localized scleroderma, systemic sclerosis and overlap syndromes. *J Eur Acad Dermatol Venereol* 2017; **31**: 1401–1424.
- Knobler R, Moinzadeh P, Hunzelmann N, et al. European dermatology forum S1-guideline on the diagnosis and treatment of sclerosing diseases of the skin, Part 2: Scleromyxedema, scleredema and nephrogenic systemic fibrosis. *J Eur Acad Dermatol Venereol* 2017; **31**: 1581–1594.
- Gambichler T, Schmitz L. Ultraviolet al phototherapy for fibrosing conditions. *Front Med (Lausanne)* 2018; **5**: 237.
- Silny W, Osmola-Mankowska A, Czarnecka-Operacz M. Eosinophilic fasciitis: a report of two cases treated with ultraviolet al phototherapy. *Photodermatol Photoimmunol Photomed* 2009; **25**: 325–327.
- Au Eong DTM, Cronin O, Biswas A, McKay ND. Ultrasound in the diagnosis and monitoring of eosinophilic fasciitis. *Rheumatology (Oxford)* 2021; **60**: e107–e108.
- Arkachaisri T, Vilaiyuk S, Torok KS, Medsger TA Jr. Development and initial validation of the localized scleroderma skin damage index and physician global assessment of disease damage: a proof-of-concept study. *Rheumatology (Oxford)* 2010; **49**: 373–381.
- Lis-Święty A, Skrzypek-Salamon A, Ranzos-Janicka I, Brzezińska-Wcisło L. Associations between disease activity/severity and damage and health-related quality of life in adult patients with localized scleroderma—a comparison of LoSCAT and visual analogue scales. *J Clin Med* 2020; **9**: 756.
- Teske NM, Jacobe HT. Using the localized scleroderma cutaneous assessment tool (LoSCAT) to classify morphea by severity and identify clinically significant change. *Br J Dermatol* 2020; **182**: 398–404.
- Gambichler T, Majert J, Pljakic A, Rooms I, Wolf P. Determination of the minimal erythema dose for ultraviolet al radiation. *Br J Dermatol* 2017; **177**: 238–244.
- Oh JH, Karadeniz F, Lee JI, Kim HR, Seo Y, Kong CS. Antiphotaging effect of (2' S)-columbianetin from *Corydalis heterocarpa* in UVA-irradiated human dermal fibroblasts. *Appl Sci* 2020; **10**: 2568.
- Pianigiani E, Tognetti L, Ierardi F, et al. Assessment of cryopreserved donor skin viability: the experience of the regional tissue bank of Siena. *Cell Tissue Bank* 2016; **17**: 241–253.
- Tognetti L, Pianigiani E, Ierardi F, et al. Current insights into skin banking: storage, preservation and clinical importance of skin allografts. *J Biorepos Sci Appl Med* 2017; **5**: 41–56.
- Murphy G, Nagase H. Progress in matrix metalloproteinase research. *Mol Aspects Med* 2008; **29**: 290–308.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–254.

- 21 Tull R, Hoover WD 3rd, De Luca JF, Huang WW, Jorizzo JL. Eosinophilic fasciitis: a case series with an emphasis on therapy and induction of remission. *Drugs Context* 2018; **7**: 212529.
- 22 Kroft EB, Berkhof NJ, van de Kerkhof PC, Gerritsen RM, de Jong EM. Ultraviolet A phototherapy for sclerotic skin diseases: a systematic review. *J Am Acad Dermatol* 2008; **59**: 1017–1030.
- 23 Weber HO, Schaller M, Metzler G, Roecken M, Berneburg M. Eosinophilic fasciitis and combined UVA1 – retinoid – corticosteroid treatment: two case reports. *Acta Dermatovenereologica* 2008; **88**: 304–306.
- 24 Mazori DR, Femia AN, Vleugels RA. Eosinophilic fasciitis: an updated review on diagnosis and treatment. *Curr Rheumatol Rep* 2017; **19**: 74.
- 25 Mango RL, Bugdayli K, Crowson CS, et al. Baseline characteristics and long-term outcomes of eosinophilic fasciitis in 89 patients seen at a single center over 20 years. *Int J Rheum Dis* 2020; **23**: 233–239.
- 26 Wang F, Garza LA, Cho S, et al. Effect of increased pigmentation on the antifibrotic response of human skin to UV-A1 phototherapy. *Arch Dermatol* 2008; **144**: 851–858.
- 27 Gambichler T, Skrygan M, Tomi NS, Breuksch S, Altmeyer P, Kreuter A. Significant downregulation of transforming growth factor-beta signal transducers in human skin following ultraviolet-A1 irradiation. *Br J Dermatol* 2007; **156**: 951–956.
- 28 Mondal S, Prosad Goswami R, Sinha D, Ghosh A. Ultrasound is a useful adjunct in diagnosis of eosinophilic fasciitis. *Rheumatology (Oxford)* 2015; **54**: 2041.
- 29 Sulli A, Ruaro B, Smith V, et al. Subclinical dermal involvement is detectable by high frequency ultrasound even in patients with limited cutaneous systemic sclerosis. *Arthritis Res Ther* 2017; **19**: 1–7.
- 30 Tognetti L, Liso FG, Nazzaro G, et al. *Ultrasound technology in practical dermatology*. Berlin, Germany: Springer Nature; 2020. 211–218.
- 31 Tognetti L, Cinotti E, Perrot JL, et al. Preliminary experience of the use of high-resolution skin ultrasound for the evaluation of extrathyroidal manifestations of Graves' disease and response to UVA-1 phototherapy. *Photodermatol Photoimmunol Photomed* 2019; **35**: 129–131.
- 32 Tognetti L, Filippou G, Bertrando S, et al. Subcutaneous fat necrosis in a newborn after brief therapeutic hypothermia: ultrasonographic examination. *Pediatr Dermatol* 2015; **32**: 427–429.
- 33 Tognetti L, Cinotti E, Tonini G, et al. New findings in non-invasive imaging of cutaneous endometriosis: dermoscopy, high-frequency ultrasound and reflectance confocal microscopy. *Skin Res Technol* 2018; **24**: 309–312.
- 34 Arndt S, Lissner C, Unger P, Bäumler W, Berneburg M, Karrer S. Biological effects of a new ultraviolet al prototype based on light-emitting diodes on the treatment of localized scleroderma. *Exp Dermatol* 2020; **29**: 1199–1208.
- 35 Dong KK, Damaghi N, Picart SD, et al. UV-induced DNA damage initiates release of MMP-1 in human skin. *Exp Dermatol* 2008; **17**: 1037–1044.
- 36 Breuckmann F, Stuecker M, Altmeyer P, Kreuter A. Modulation of endothelial dysfunction and apoptosis: UVA1-mediated skin improvement in systemic sclerosis. *Arch Dermatol Res* 2004; **296**: 235–239.
- 37 Wlaschek M, Heinen G, Poswig A, Schwarz A, Krieg T, Scharffetter-Kochanek K. UVA-induced autocrine stimulation of fibroblast-derived collagenase/MMP-1 by interrelated loops of interleukin-1 and interleukin-6. *Photochem Photobiol* 1994; **59**: 550–556.
- 38 Jinnin M, Yamamoto T, Asano Y, et al. Diagnostic criteria, severity classification and guidelines of eosinophilic fasciitis. *J Dermatol* 2018; **45**: 881–890.
- 39 Schiener R, Behrens-Williams SC, Gottlöber P, Pillekamp H, Peter RU, Kerscher M. Eosinophilic fasciitis treated with psoralen-ultraviolet A bath photochemotherapy. *Br J Dermatol* 2000; **142**: 804–807.
- 40 Moy AP, Maryamchik E, Nikolskaia OV, Nazarian RM. Th1- and Th17-polarized immune infiltrates in eosinophilic fasciitis-A potential marker for histopathologic distinction from morphea. *J Cutan Pathol* 2017; **44**: 548–552.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Densitometric analysis of zymography gels.

Supernatants protein content was evaluated with the Bradford method;²⁵ 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel (PAG) was copolymerized with 0.1% gelatin and 20 mg of protein of each sample was loaded into each well under nondenaturing conditions and run under a constant current (25 mA). After electrophoresis, the gel was rinsed in 2.5% Triton X-100 and 50 mmol/l Tris–HCl (pH 7.5) and then incubated overnight in an activation buffer (50 mmol/l Tris, pH 7.5, 0.15 mol/l NaCl, 10 mmol/l CaCl₂, and 1% Triton X-100). The gel was stained with 0.25% comassie brilliant blue, 50% methanol, and 10% acetic acid and de-stained with 30% methanol and 10% acetic acid [AU: arbitrary units of integrated density].