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CLINICAL REPORT

Three Randomised Phase I/IIa Trials of 5% Cis-urocanic Acid Emulsion Cream in Healthy Adult Subjects and in Patients with Atopic Dermatitis

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New treatment modalities are needed in atopic dermatitis. We evaluated the pharmacokinetics, safety, tolerability, and efficacy of topical cis-urocanic acid (cis-UCA) cream in randomised vehicle-controlled double-blinded clinical trials. The subjects received 5% cis-UCA emulsion cream and control vehicle on volar forearms after right–left randomisation. Study 1: 16 healthy subjects received one dose on the skin and, a week later, on DMSO-irritated skin. Study 2: 16 healthy subjects received 2 daily doses for 10 days. Study 3: 13 patients with mild to moderate disease were treated on selected skin lesions twice daily for 28 days. Study treatments were well tolerated. cis-UCA remained close to endogenous levels in plasma and urine. cis-UCA reduced transepidermal water loss (TEWL) both in healthy subjects and in the patients. Eczema area severity index and physician's global assessment improved from baseline with both treatments. cis-UCA cream improved skin barrier function and suppressed inflammation in the human skin. ***Key words: randomised controlled trial; tolerability; skin barrier function; transepidermal water loss; skin inflammation; erythema.***

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Eczema and atopic dermatitis (AD) are major health problems with prevalence estimates as high as 1/3 of the population depending on the country studied and the diagnostic criteria used (1). In all types of dermatitis, local inflammation is always involved. The central role of the inflammation component is obvious when considering the therapeutic options for dermatitis. The most effective local drug therapy consists of anti-inflammatory and immunosuppressive agents used in affected skin lesions. While topical steroids are still the most widely used therapy, novel topical calcineurin inhibitors have gained popularity in the treatment of AD (2).

Urocanic acid (UCA) is an endogenous molecule of the skin, an important component of hygroscopic and pH-regulating materials called natural moisturising factors (3–5), and a photoprotective agent (6). Dehydration of the skin activates filaggrin proteolysis into histidine (3), the precursor of trans-UCA. Epidermal UCA concentrations correlate strongly negatively with AD severity (4). Since cis-UCA suppresses cell-mediated (7–9) and innate immunity (10, 11), and acute or subacute skin inflammation (12), we wanted to investigate the use of cis-UCA cream formulation in the treatment of AD. Three randomised vehicle-controlled clinical trials were performed, 2 in healthy adult subjects and one in adult patients with mild to moderate AD. We envisioned that cis-UCA could show a positive local anti-inflammatory effect and improve skin barrier function without significant adverse effects.

MATERIAL AND METHODS

Investigational products

The investigational products were 5% (w/w) cis-UCA emulsion cream (BioCis Pharma, Turku, Finland) and the same vehicle emulsion cream base (Orion, Espoo, Finland). The cream base contains aqua, decyl oleate, cetearyl alcohol, glycerin, sodium cetearyl sulphate, and methyl paraben. Both products were pH 6.5.

Randomisation and blinding

In all 3 studies, the study subjects, the investigators, and the study site personnel were blinded for the identity of the treatments. All subjects were treated with both a cis-UCA and control vehicle product on the volar aspect of the forearm and randomised for the right and left arm. The products were packed in identical tubes labelled for either arm for each subject number. Separate randomisation lists were prepared for consequent phases of a study. Randomisation was balanced by gender for studies 2 and 3. Randomisation was performed by computer-generated (SAS® System, Cary, NC, USA) lists by a randomisation expert with no clinical involvement in the trials.

Participants and study design

The trials were prospectively registered in the European Clinical Trials Database with numbers 2007-006705-24 (Study 1), 2008-004428-22 (Study 2), and 2008-005075-10 (Study 3) and conducted in accordance with Good Clinical Practice Guidelines and the Declaration of Helsinki, with approval granted by the Ethics Committee of the Hospital District of Southwest Finland. The subjects were recruited using advertisements in a news-

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paper, on bulletin boards, and online. The given information and a register of potential healthy subjects (CRST, Turku, Finland) were approved by the ethics committee before recruitment. Study procedures were implemented after obtaining a written informed consent from each study subject. Demographical details of the subjects included have been summarised in Table SI[1]. All study procedures were performed in a single inpatient unit at CRST in Turku, Finland, between October and May in 2008 and 2009 (Appendix S1[1]). The studies ended as the targeted number of subjects was achieved.

Study 1. This was a Phase I study to evaluate the pharmacokinetics, safety, tolerability, and preliminary efficacy of cis-UCA in healthy subjects. Sixteen out of 18 screened male volunteers with no past or current skin disease were included in the study (Table SI[1], Fig. S2[1]; See Appendix S1[1]: Supporting information for full disclosure of inclusion and exclusion criteria). The study was composed of 2 phases (Fig. S1[1]). In the 1st phase, the subjects received a single dose of the 5% cis-UCA cream (0.7 mg cis-UCA kg^{-1}) on one volar forearm and control vehicle on the other forearm over a skin area of 48 cm^2 (6 × 8 cm). Pharmacokinetic blood samples collected until 24 h and urine until 72 h were analysed by using a validated LC-MS/MS method under Good Laboratory Practice. Tolerability was evaluated by visual skin reaction severity (VSS) scoring for erythema, skin swelling, formation of papules, formation of vesicles or bullae, and scaling (each graded as 0–3) until 12 h after treatments and again at 24 h. Eligibility to attend the 2nd phase was evaluated on day 8 (Fig. S1[1] and Appendix S1[1]). The 2nd phase started on day 8 by inducing acute skin irritation with 20 µl of 100% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) in 10 8-mm aluminium occlusion chambers (5 cm^2 in total; Epitest, Tuusula, Finland) attached for 5 min at a skin site different from that used in the 1st phase on both lower volar forearms. Five min after removal of the chambers, the smallest dose of cis-UCA (44.1 mg/0.9 g) and control vehicle (0.9 g) administered in the 1st phase was applied over a 48-cm^2 skin area covering 5 DMSO spots, leaving 5 DMSO spots per arm as untreated controls. Any unabsorbed cream was wiped off at 15 min after application. Skin erythema (UV-Optimize Matic 555; Matic, Herlev, Denmark), TEWL (VapoMeter; Delfin Technologies, Kuopio, Finland), and VSS scoring were assessed in the DMSO spots until 4.5 h. Tolerability of the products was evaluated by VSS scoring in the non-DMSO-treated skin until 4 h. The subjects were observed until 4.5 h at the study centre, and thereafter, they visited the study centre one and two weeks later.

Study 2. Pharmacokinetics, safety and tolerability of cis-UCA were evaluated in a repeated-dose Phase I study with healthy adult subjects. Sixteen out of 26 screened male and female subjects (Table SI[1]) with no past or current skin diseases were included (Appendix S1[1]). All included subjects completed the study (Fig. S2[1]). The subjects received 5% cis-UCA cream twice daily (0.7 mg cis-UCA kg^{-1} day^{-1}) on one volar forearm and control vehicle on the other forearm for 10 days (Fig. S1[1]). The morning doses were applied at the study centre and pre-weighed evening doses were self-administered at home. After the morning dose on day 10, safety and skin tolerability were evaluated as in Study 1, and pharmacokinetic blood samples were collected until 24 h and urine fractions for 72 h.

Study 3. The 3rd clinical trial was a vehicle-controlled, repeated and multiple-dose Phase I/IIa study to evaluate pharmacokinetics, safety, tolerability, and efficacy of cis-UCA in adult subjects with chronic, mild to moderate AD treated for up to 28 days. The study was composed of a pharmacokinetic phase for 10 days followed by an extension phase for 18 days (Fig. S1[1]). Altogether 13 out of 42 screened subjects were included in the study (Fig. S2[1], Table SI[1] and Appendix S1[1]). Two screening visits within 4 weeks before treatments with at least 2 weeks apart were performed to evaluate variations in skin symptoms. AD was classified as moderate (10/13) or mild (3/13). Representative symmetrically affected volar forearm skin sites were dosed with 5% cis-UCA (0.7 mg cis-UCA kg^{-1} day^{-1}) on the forearm between antecubital fossa and the wrist and control vehicle on the other forearm twice daily for 10 days. The morning doses were applied at the study centre and pre-weighed evening doses at home. After the morning dose on day 10, safety and skin tolerability were evaluated as in Study 1, and pharmacokinetic blood samples were collected until 24 h and urine fractions for 72 h. Subjects with no safety concerns in the pharmacokinetic phase were allowed to continue in the extension phase starting on day 14 for up to 18 days, totalling 28 days of cis-UCA treatment. In the extension phase, the subjects received half a dose of both treatments (0.35 mg cis-UCA kg^{-1} day^{-1}) on their forearms twice daily. The subjects self-administered the doses at home after the 1st dose on day 14 by using a disposable dosing scale card. VSS was assessed for evaluation of tolerability and as an indicator of treatment efficacy. Skin erythema and TEWL were measured on several study days. In addition, physician's global assessment (PGA) and total-body eczema area severity index (EASI) were performed on several study days. The subjects documented the administration of treatments, possible adverse effects (AEs), concomitant medications, and any deviations from the instructions in a dedicated diary.

Statistical methods and determination of sample size

Data management and statistical analyses were planned and performed by 4Pharma Ltd, Turku, Finland. Descriptive statistics by treatment group and study day were provided to summarise the study results. For safety and tolerability, non-parametric Wilcoxon signed rank test was used. Standard statistical methods for paired data, such as paired *t*-test, non-parametric Wilcoxon signed-rank test, and repeated measurements analysis of covariance (RM ANCOVA) were used for efficacy variables. The number of subjects planned to be included in the studies was based on clinical considerations only. Formal sample size calculations were therefore not performed. (See Appendix S1[1] for more details).

RESULTS

Pharmacokinetics

In Studies 1 and 2, cis-UCA concentrations in plasma and urine remained below the analytical detection threshold (0.2 and 2 µg/ml, respectively) at all times, corresponding to endogenous base-line levels. Also in Study 3, cis-UCA was not detected in plasma and urine with the exception of 2 AD patients, who had single low concentrations (3.5 and 4.8 µg/ml) of cis-UCA in urine samples taken before dosing on day 10. The low concentrations suggest very low or negligible systemic exposure to cis-UCA. No pharmacokinetic parameters were calculated.

Tolerability and efficacy in Study 1

The tolerability of cis-UCA was comparable to control vehicle. There were no serious or other significant AEs

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(Appendix S1[1]). No visual skin reaction severity (VSS) score differed from zero.

cis-UCA decreased the DMSO-induced elevation of TEWL at all measured time points compared to control vehicle (Fig. 1). At the peak time point observed at 30 min after DMSO occlusion, the control vehicle increased TEWL, which was statistically significantly inhibited by cis-UCA ($p=0.0056$, paired t-test) (Fig. 1b). Over the whole treatment period (0.5–4.5 h), the cis-UCA vs. control vehicle treatment difference was –1.84 units (95% CI –3.25 to –0.42; $p=0.014$, RM ANCOVA treatment effect). Erythema was decreased in both treatments but no significant differences were found between the treatments over the whole treatment period. Erythema was reduced by cis-UCA at 1 h compared to control vehicle ($p=0.046$, paired t-test). No statistically significant differences were found in the VSS evaluation between the treatments.

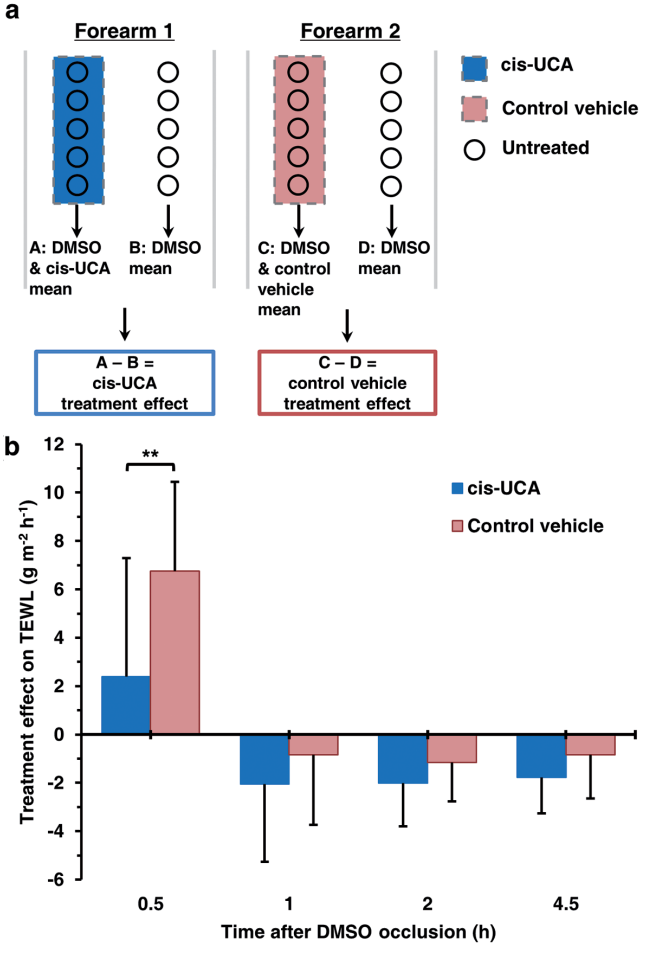

Fig. 1. Effect of study treatments on TEWL after DMSO irritation of the healthy skin (Study 1). (a) The volar forearm skin was treated with DMSO in 10 occlusion chambers in each forearm for 5 min and treated with either cis-UCA or control vehicle in a randomised double-blind fashion. (b) TEWL was measured in all spots at several time points until 4.5 h (mean ± SD). Statistically significant difference between treatments (paired t-test) is shown; $^{**}p<0.01$.

Tolerability in Study 2

Study treatments were well tolerated. There were no serious or other significant AEs and no discontinuations. Out of 17 treatment-emergent AEs reported (14 mild AEs/6 subjects, 3 moderate/3 subjects), 6 were skin-related and mild; however, none of them had causal relationship to cis-UCA (Appendix S1[1]). Erythema was scored as mild 17 times on the volar forearm with cis-UCA application and 21 times with control vehicle. Formation of papules was reported 3 times with cis-UCA treatment and 5 times with control vehicle. No accumulation of VSS findings to any time point assessed was observed, and there were no statistically significant differences between the treatments.

Tolerability and efficacy in Study 3

Study treatments were well tolerated. There were no serious or other significant AEs and no discontinuations due to AEs. Thirty AEs were reported by 10 subjects (9 events/3 males, 21 events/7 females); 7/22 treatment-emergent AEs were considered moderate and 15/22 mild. The most frequent treatment-emergent skin-related AEs were pruritus (5 events/1 subject) and worsening of AD (2 events/2 subjects). There were 6 treatment-related AEs in one female subject; 4 occasions (3 moderate and 1 mild) of pruritus and 2 occasions of mild burning sensation on the investigational areas on both treatment sides. All other treatment-emergent AEs were considered not related to the study medications (Appendix S1[1]).

In intent-to-treat analysis ($n=13$), cis-UCA decreased TEWL significantly more than control vehicle over the whole treatment period ($p=0.024$, RM ANCOVA treatment effect) (Fig. 2a). When compared to baseline TEWL on day 1, cis-UCA caused a significant mean decrease ($p=0.043$) whereas no significant decrease was observed with control treatment (Fig. 2a). Also, significantly lower TEWL values in skin areas treated with cis-UCA were obtained on days 10 ($p=0.020$, paired t-test) and 28 ($p=0.008$), as compared to control vehicle. For skin erythema, the decrease from baseline was significant over the whole treatment period for cis-UCA ($p=0.012$, RM ANCOVA) but not for control vehicle or between the treatments (Fig. 2a). Further, the decrease from baseline erythema with cis-UCA treatment was statistically significant on days 10 ($p=0.023$), 21 ($p=0.009$), and 28 ($p=0.017$), whereas no significant differences were observed with control vehicle.

The measured erythema data revealed clear division of the study population into high- and low-erythema subgroups at baseline. Although not pre-defined in the study protocol, statistical re-analysis by erythema subgroup was therefore considered of interest after selecting the mean erythema level + 1 SD in the skin of healthy individuals (22.1 erythema units in Study

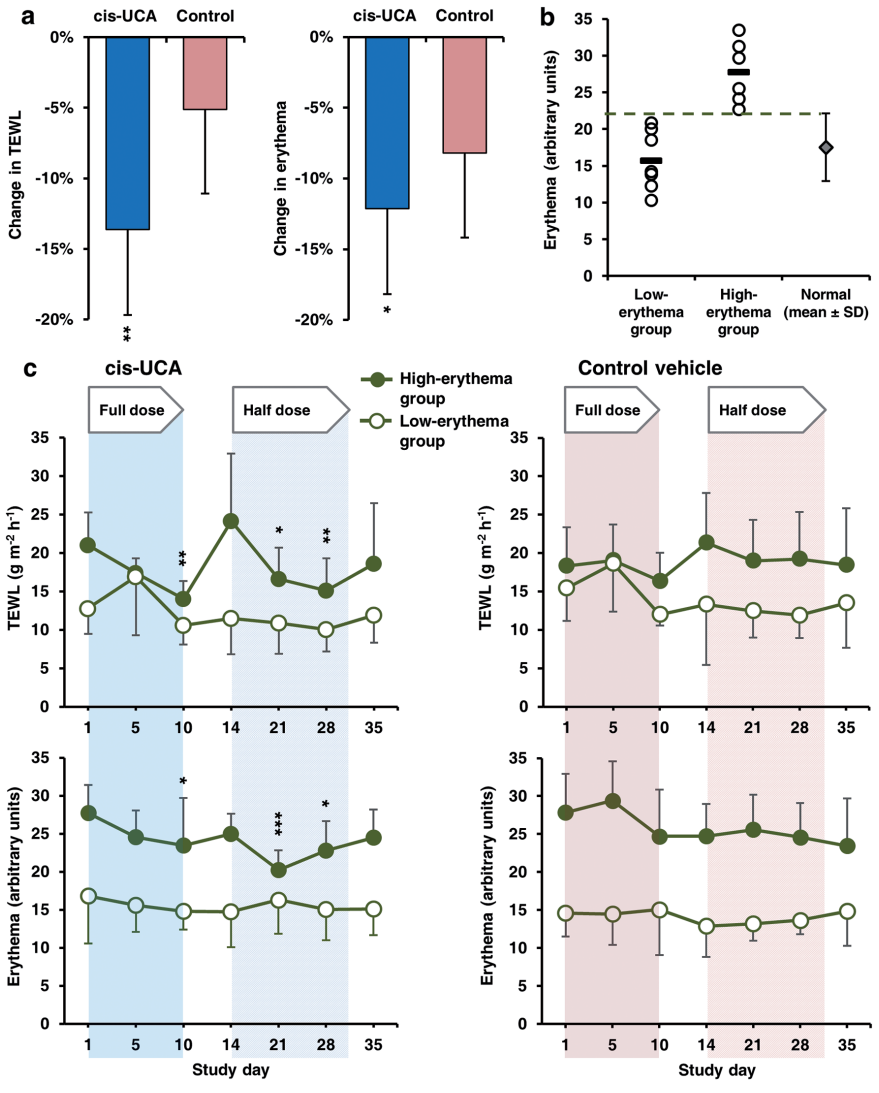

Fig. 2. Treatment of the atopic skin with cis-UCA (Study 3). (a) The overall change (% mean decrease ± SD, $n = 13$) in TEWL and skin erythema from baseline (day 1) after twice daily treatment with 5% cis-UCA emulsion cream and control vehicle. (b) Division of the study population into high- and low-erythema subgroups at baseline for statistical re-analysis. The "Normal" erythema mean + 1 SD is from Study 1 in healthy male individuals ($n = 16$). Black bars denote the mean of data points. (c) Efficacy of cis-UCA treatment on skin barrier function (TEWL) and skin erythema in high ($n = 6$) and low erythema ($n = 7$) subgroups of AD patients. Statistically significant change from the baseline (paired *t*-test) is shown; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

1; $n = 16$) as the division value (Fig. 2b). In the high-erythema subgroup (≥ 22.1 erythema units; $n = 6$), TEWL decreased highly significantly over the whole treatment period with cis-UCA as compared to control vehicle ($p = 0.002$). Also the mean decrease from the baseline was significant for cis-UCA ($p = 0.017$) but not for control. TEWL decreased significantly in the cis-UCA-treated forearm skin at days 21 ($p = 0.024$, paired *t*-test) and 28 ($p = 0.010$) in comparison to control vehicle and at days 10 ($p = 0.002$), 21 ($p = 0.037$), and 28 ($p = 0.007$) as compared to baseline, whereas control vehicle treatment did not cause statistically significant changes (Fig. 2c). Likewise, skin erythema decreased highly significantly from baseline over the whole treatment period with cis-UCA as compared to control vehicle ($p = 0.003$). The decrease from baseline was statistically significant on days 10 ($p = 0.023$), 21 ($p < 0.001$), and 28 ($p = 0.015$) for cis-UCA (Fig. 2c). In the low-erythema (< 22.1 erythema units at baseline; $n = 7$) subgroup, no significant differences in TEWL or erythema were observed at any time point or over the whole treatment period (Fig. 2c). For both treatments, significant improvement was seen in EASI and PGA scores; the differences between cis-UCA and the control vehicle were not, however, statistically significant.

DISCUSSION

In this report were presented results from 3 separate randomised and vehicle-controlled clinical trials with topical cis-UCA treatment in human subjects. The primary objective in the studies was to evaluate the pharmacokinetics of cis-UCA after topical dosing in adult subjects. Based on knowledge of preclinical pharmacokinetics showing rapid and complete renal clearance of cis-UCA from the circulation in an unchanged form (Laihia and Leino, unpublished observations) and considering the limited skin area treated with cis-UCA in the human studies, low systemic concentrations were expected. Pharmacokinetic analyses confirmed that systemic exposure to cis-UCA remained mainly at endogenous (undetectable) levels at all time points

studied even in AD patients treated twice daily with 0.7 mg cis-UCA kg^{-1} day^{-1}. There was no indication of systemic accumulation of cis-UCA after repeated topical administration.

Due to the very low or negligible systemic exposure, the secondary objectives of the studies, especially systemic safety and tolerability, were expected to create little concern. In all assessments, the 5% cis-UCA emulsion cream was found well tolerated both locally on the skin and systemically. Several previous studies in a total of 80 adult subjects exposed to single or repeated doses of topical cis-UCA formulations (9, 14–18) also on large skin areas (9) confirm our tolerability findings. In each of these studies, the positive anti-inflammatory response to cis-UCA treatment could be verified and no adverse effects or treatment-related local or systemic side effects were reported. It can therefore be concluded that treatment of the healthy and affected skin (after experimental irritation by DMSO and in patients with AD) with cis-UCA emulsion cream in adult subjects for up to 28 days is well tolerated.

The preliminary efficacy of cis-UCA emulsion cream was delineated as a secondary objective in 2 of the studies. In Study 1, the efficacy was investigated in healthy adult subjects with experimentally induced acute skin irritation. DMSO is a virtually non-toxic skin penetration enhancer that causes transient skin irritation, leukocyte infiltration, pore formation, and increasing TEWL (19–23). In animal studies, the 5% cis-UCA cream significantly suppressed DMSO-induced mouse ear swelling at all time points evaluated when compared to control vehicle (12). The present results revealed significantly larger reduction in TEWL during the observation period in cis-UCA-treated skin than in control vehicle-treated skin (Fig. 1), while skin erythema was reduced significantly faster compared to control vehicle at the peak time point (1 h) only. Both the present results (Fig. 1) and mouse skin studies (12) show that some of the constituents of the vehicle cream may cause mild and transient aggravation of the skin reaction which is attenuated by cis-UCA. However, beause TEWL is an indirect indicator of the skin barrier function, the effect of cis-UCA on DMSO-induced impairment of skin barrier function remains to be elucidated with more direct methods such as electron microscopy of the lipid lamellae. In Study 3, the overall efficacy results indicated superiority of the 5% cis-UCA emulsion cream over control vehicle in improving skin barrier function (measured as TEWL) and in decreasing skin redness in subjects with mild to moderate AD. Significant improvement was observed already 10 days after the start of cis-UCA treatment. Significant improvement in PGA and EASI in the treatment area was also observed, but without statistically significant differences between treatments, obviously due to a relatively high vehicle effect, short treatment period and small lesion size used, which makes it difficult to produce significant changes in EASI; it is noteworthy that while the Aqualan® emulsion cream used as the control vehicle may cause transient aggravation of skin symptoms, it is an emollient base cream product recommended for dry skin and atopic skin care. All subjects received both cis-UCA and control vehicle treatments. Therefore, any possible difference between the 2 cream compositions, such as consistency or skin absorption properties could not affect the primary endpoint, pharmacokinetics, the device measurements, or the evaluation by investigators who remained blinded; no self-reported efficacy parameters were recorded either. As revealed by sub-analysis of the 6 AD patients with active skin inflammation in the treatment area, erythema and TEWL improved significantly with cis-UCA but not with control vehicle (Fig. 2c). It is obvious that the intent-to-treat analysis was biased by patients with a non-inflamed skin condition, and therefore an *ad hoc* analysis of the data was justified. The results also suggest that instrumental skin assessment methods, such as measurement of skin redness by skin reflectance and skin barrier function by TEWL, are more sensitive to record treatment effects than subjective visual scoring methods.

In AD lesions and other skin inflammation conditions, the skin barrier function is impaired, increasing TEWL and reducing skin hydration. After acute permeability barrier disruption, the expression of proinflammatory cytokines in the epidermis increases, attracting and stimulating inflammatory cells at the reaction site. In the acute phase, neutrophils dominate, whereas T cells become prevalent in more chronic inflammation. Recent investigations suggest that cis-UCA can inhibit the extracellular production of reactive oxygen species (10, 11, 24) and inflammatory cytokines (24, 25) from leukocytes and epithelial cells. This is believed to take place by a protodynamic mechanism of action based on a favourable pK_a value of cis-UCA in physiological conditions (26) and involving intracellular acidification as earlier demonstrated in cancer cells (27–30). In the acidic epidermal environment and topical formulation, cis-UCA could thus inhibit the function of inflammatory leukocytes and keratinocytes, restricting local tissue damage and helping reduce the inflammatory response.

The 3 clinical studies indicate that the 5% cis-UCA emulsion cream is well tolerated in adult subjects. The pharmacokinetic results suggest very low or negligible systemic exposure to cis-UCA. Due to the exploratory nature of the present studies with a relatively small number of subjects, the findings should be interpreted with caution. However, reduction in TEWL by cis-UCA strongly indicates improved skin barrier function, a crucial factor in inflammatory skin disorders. This aspect is currently under further investigation in a larger number of adult subjects with moderate or severe AD (ClinicalTrials.gov NCT01320579).

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Conflicts if interest: The 3 studies were funded by BioCis Pharma Ltd. The authors LP, LL, and JKL were employees of BioCis Pharma Ltd at the time the studies were conducted. LL and JKL are also shareholders and patent inventors for BioCis Pharma Ltd.

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