Appendix S1. SUPPORTING INFORMATION

STUDY 1: Double-blind, vehicle-controlled, single centre, two-period, Phase I study to investigate pharmacokinetics, safety and tolerability of a single topical dose of 5% cis-urocanic acid, and to investigate effects of a single topical dose of 5% cis-urocanic acid in experimentally induced acute irritant dermatitis in healthy adult male volunteers

Inclusion criteria

1. Informed consent obtained prior to any screening procedures.
2. White skinned male between 18–45 years of age.
3. Good general health ascertained by medical history, physical examination, ECG recording and laboratory determinations, showing no signs of clinically significant findings.
4. Healthy skin.
5. Body weight 60–100 kg; body mass index (BMI) 18–30 kg/m^2.
6. Supine blood pressure (BP) after 3 min rest: systolic 100–150 mmHg, diastolic 60–90 mmHg, and supine heart rate (HR) after at least 3 min rest: 40–90 beats/min.
7. Non-smoker.

Exclusion criteria

1. History of significant skin disease (e.g. any skin disease requiring hospitalisation), or skin manifestations of allergic illness or other dermatologic condition that would interfere with the trial assessments or compromise the subject's safety according to the opinion of the investigator.
2. Present symptoms of atopic dermatitis, solar dermatitis, ichthyosis or skin dryness of any other cause, or any other current skin disease according to the opinion of the investigator.
3. Sensitive skin of any reason (subject's personal opinion) (subjects who often develop non-specific skin reactions to various preparations or from unknown reasons).
4. Tattoos on the volar side of either forearm.
5. Recent history of a sunny holiday or solarium use within 2 months (60 days) before beginning of period I, or planning such during the study or within 30 days after the study.
6. Damaged skin at the test site (e.g., uneven skin pigmentation, numerous freckles, scars or other disfigurations) or clinical signs or symptoms of skin irritation (e.g. pruritus, burning, erythema).
7. Allergy to cis-UCA, or any constituents of Aqualan® (decyl oleate, cetearyl alcohol, glycerine, sodium cetearyl sulphate and methylparaben).
8. History of any cancer or current cancer.
9. Use of prescription drugs within 14 days prior to dosing or over the counter medication within 7 days prior to dosing.
10. Donation of blood or participation in another drug study within 60 days before the 1st product administration in this study.
11. Any medical condition (such as renal impairment) which might significantly alter the absorption, distribution or excretion of cis-UCA.
12. Any clinically significant laboratory test result (including positive tests for HIV and hepatitis B or C).
13. Excessive use of alcohol (on average > 24 units per week, unit = 4 cl spirits or equivalent).
14. Suspected current use of illicit drugs (medical history, physical examination, screening laboratory determinations).
15. Clinically significant illness during the 4 weeks prior to the 1st dose administration (as determined by the investigator).
16. Any other condition that in the opinion of the investigator would interfere with the evaluation of the study results or constitute a health hazard for the subject.
17. Unwillingness or doubtful capacity to comply with the protocol.
18. Doubtful availability, in the opinion of the investigator, to complete the study.
19. Poor peripheral venous access.
20. The length of the volar forearm is < 22 cm (measured from the crook of the arm to the wrist).

Instructions concerning lifestyle and concomitant medications

At the screening visit, the subjects were advised to comply with the following instructions:

1. The subjects were to refrain from strenuous physical exercise for 48 h before the screening visit, end-of-study visit, and before 1st treatment administration until the end of blood sampling (period I).
2. The subjects were to refrain from the use of prescription drugs within 14 days prior to dosing or over the counter medication within 7 days prior to dosing (including herbal remedies, trace elements, vitamins, dietary supplements) and during the study. Paracetamol may have been taken for occasional pain.
3. The subjects were to refrain from consuming alcohol products for 48 h before the screening visit, end-of-study visit, and each treatment administration until the end of blood sampling and urine collection (only in period I).
4. The subjects were to have fasted for at least 10 h before each safety laboratory determination (at screening and at end-of-study visit) and before the 1st treatment administration (period I).
5. The subjects were allowed to have only meals and drinks served by study personnel at the study site while confined. Water was allowed from 4 h after the 1st administration (period I) *ad libitum*. No restrictions in water consumption were needed after the 2nd treatment (period II).
6. Restrictions concerning test areas in period I: The subjects were not allowed to touch the test areas with

fingers, clothes or by other means at least for 60 min, or even longer until all of the emulsion cream was absorbed in the skin. The subjects were not allowed to let the test area to get wet for 24 h after the administration. The test areas were not to be scrubbed or scratched during the 24 h after the treatment. In addition, sauna bath or shower was not allowed during 24 h after the administration.

7. Restrictions concerning test areas in period II: The subjects were not allowed to touch the test areas with fingers, clothes or by other means, or to let the areas to get wet during the confinement at the study site, up to approximately 4.5 h.
8. The study personnel were to strengthen the test area corners with a felt pen on days 2, 3 and 4, if needed. After that, the subjects were to strengthen the application site corners with a felt pen after the 1st treatment until day 8, if the marks were faint.
9. The subjects were to report all adverse effects (AEs) and concomitant treatments to the study personnel. Minor AEs might have been reported at visits, and significant ones as soon as possible.
10. The subjects were instructed not to participate in another clinical drug study or donate blood within 60 days after the last blood sampling of this study.
11. The subjects were to collect the urine in a container in period I, from day 1 to day 4 (until 72 h) and to bring the containers to the Phase I unit as requested.

Disposition of subjects

Of the 18 screened subjects, 16 were included in the study. One subject withdrew his consent shortly after screening visit. One subject was screened as a reserve subject, but he was not included.

The 1st subject was screened on 18 February 2008. The 1st dose was administered on 28 February 2008 and the last dose on 27 March 2008. The last subject completed the study on 16 April 2008.

Adverse events

In both periods, the study treatments were well tolerated and safe. There were no deaths, serious or other significant AEs. There were no discontinuations due to AEs.

A total of 25 AEs occurred in 11 subjects during the entire study. Seven AEs in 5 subjects occurred prior to treatment administration and 18 AEs in 8 subjects occurred after starting the study treatments. The most frequently reported treatment-emergent AEs were headache reported 4 times by 4 subjects, and upper respiratory tract infection reported 3 times by 3 subjects. All other AEs were reported by single subjects only. All AEs were mild in severity with the exception of one subject with moderate abdominal pain, assessed to be non-related to the study treatments. One subject reported 5 episodes of mild papular rash on volar arms during the study; one occasion on both volar arms was assessed to be related to the study treatments. One subject had significant findings in laboratory safety variables at the post-study visit most probably due to a preceding viral infection. Other laboratory safety findings of all subjects during the study were clinically not significant. No VSS scoring results differed from 0. In conclusion, the safety and tolerability of cis-UCA and placebo were very good and comparable.

Measurement of treatment compliance

The treatments were administered by the study personnel and the compliance is therefore 100%.

Statistical methods and determination of sample size

Descriptive statistics by study day and time point were provided to summarise the study results, including changes from baseline where applicable. Summary statistics included the number of subjects, mean, standard deviation, standard error, minimum, median and maximum for continuous variables, and frequencies and percentages for categorical variables. Standard statistical methods for paired data (e.g. paired *t*-test and Wilcoxon signed-rank test) were used for efficacy variables. Also repeated measurement models were used to characterise the effects after DMSO. No imputations were done for missing observations. No interim analyses were performed. No formal hypotheses were tested in this explorative study and as a consequence, no multiple testing adjustments were performed. A two-sided p-value of < 0.05 was considered statistically significant. All analyses and data tabulations/descriptions were done separately for period I and II.

The number of subjects planned to be included in this study was based on clinical considerations. A formal sample size calculation was therefore not performed. Sixteen healthy male volunteers were included in the study as planned in the study protocol.

STUDY 2: Double-blind, vehicle-controlled, repeated dose, single centre Phase I study to investigate pharmacokinetics, safety and tolerability of topical twice daily doses of 5% cis-urocanic acid for up to 10 days in adult healthy volunteers

Inclusion criteria

Identical to criteria used in Study 1, except for the following points;

1. White skinned male or female between 18 and 65 years of age.
2. Negative pregnancy test (premenopausal female subjects) at screening and use of adequate contraceptive measures (both male and female subjects) throughout the study and 30 days after the last cis-UCA dose;
   - Premenopausal female volunteers should be either surgically sterile or using a reliable contraception method: intrauterine device (hormonal or non-hormonal); oral combination pill or hormonal contraception patch; or two of the following: intravaginal hormonal ring, oral contraceptive containing progestin only, spermicidal foam, condom, sterilisation of male sexual partner (surgical vasectomy).

- Subjects with no current heterosexual relationship may be included according to the judgement of the investigator.
- If menopause occurred 2 years earlier at the minimum, no contraception is required for female participants, nor pregnancy tests.
- Reliable contraception for male subjects is concordant with above listed methods for females, as applicable.

3. Body weight 45–85 kg for females.
4. Non-smoker and not using any nicotine products.

Exclusion criteria

Identical to criteria used in Study 1, except for the following points;

1. History of sunny holiday or solarium use within 1 month (30 days) before beginning of study treatments, or planning such during the study or within 30 days after the study.
2. Earlier participation in a clinical study performed with cis-UCA.
3. Use of prescription drugs within 14 days prior to dosing or over the counter medication within 7 days prior to dosing. Paracetamol is allowed for occasional pain. Antihistamine is allowed for allergic symptoms. Non-steroidal nasal sprays and eye drops are also allowed.
4. Donation of blood or participation in another drug study within 60 days (males) or 90 days (females) before the 1st product administration in this study.
5. Excessive use of alcohol (on average > 24 units per week for males, and more that 16 units per weeks for females; unit = 4 cl spirits or equivalent).

Instructions concerning lifestyle and concomitant medications

At the screening visit, the subjects were advised to comply with the following instructions:

1. The subjects were to refrain from strenuous physical exercise for 48 h before the screening visit (including laboratory assessments), and end-of study visit, and before the treatment administrations on days 1 and day 10, and until the end of blood sampling after day 10 (i.e., day 11 morning).
2. The subjects were to refrain from the use of prescription drugs within 14 days prior to dosing or over the counter medication within 7 days prior to dosing (including herbal remedies, trace elements, vitamins, dietary supplements) and during the study. Paracetamol may have been taken for occasional pain. In addition, antihistamines and use of non-steroidal nasal sprays and eye drops were allowed for allergic symptoms.
3. The application area was to remain intact and it should not be touched, e.g. by a finger, for at least 2 h after each application.
4. The subjects were to refrain from taking showers more than twice daily during the study medication until the end of blood sampling on day 10 (exceptions in point 10).
5. The subjects were to take their morning shower (if any) before their visit to the Phase I unit. Likewise, they were to take their evening shower (if any) before the application of the evening dose (exceptions in point 10).
6. The subjects were to refrain from sauna bath during the study medication until the end of blood sampling after day 10.
7. The subjects were to refrain from consuming alcohol products for 48 h before the screening visits, before the day 1 and 10 visit, and until the end of blood sampling and urine collection after day 10, and before the end-of-study visit.
8. The subjects were to have fasted for at least 10 h before each safety laboratory determination (at screening and at end-of-study visit) and before the 1st treatment administration on days 1 and 10.
9. The subjects were allowed to have only meals and drinks served by study personnel at the study site while confined for PK assessment on study day 10. Water was allowed from 4 h on study day 10 *ad libitum*. No restrictions in water consumption were needed on other study days.
10. Restrictions concerning the treatment areas for PK assessments on study day 10: The subjects were not allowed to touch the treatment areas with fingers, clothes or by other means at least for 2 h or even longer until all of the emulsion cream was absorbed in the skin. The subjects were not allowed to let the treatment area to get wet for approximately 12 h after the administration. The treatment areas were not to be scrubbed or scratched during the 12 h after the treatment. In addition, shower was not allowed during 12 h after the administration.
11. The subjects were to wash their hands before and after the application of the study treatments with mild soap and water, carefully avoiding that the treatment area will get wet.
12. The subjects were to report all AEs and concomitant treatments to the study personnel. Minor AEs might be reported at visits, and significant AEs as soon as possible.
13. At home study subjects were to fill the diary (AEs, concomitant medications, other deviations, evening dose application) starting from the screening visit until the end-of study visit.
14. The subjects were to collect the urine in containers from day 10 to day 13 (until 72 h after the last cis-UCA dose) and to bring the containers to the Phase I unit as requested.
15. The subjects were instructed not to participate in another clinical drug study or donate blood within 60 days (males) or 90 days (females) after the last treatment dose of this study.
16. The subjects were instructed to use adequate contraceptive measures during the study and at least 30 days after the last cis-UCA dose.

Disposition of subjects

Of the 26 screened subjects, 16 were included in the study. The reasons for exclusions for the 10 subjects were as follows: decreased blood haemoglobin value (2 subjects), low blood pressure, high blood pressure, numerous scars on the left arm at the test site, uneven pigmentation at the test site, tattoo on the volar side of right forearm, increased plasma

alanine transferase (ALAT) value, bath therapy to lichen ruben in anamnesis, and sunny holidays planned within 30 days after the study. The subjects were instructed to further examinations, if clinically judged.

The 1st subject was screened on 7 October 2008. The 1st dose was administered on 13 October 2008 and the last dose on 19 November 2008. The last subject completed the study on 5 December 2008.

Adverse events

Study treatments were well tolerated and safe. There were no deaths, serious or other significant AEs. There were no discontinuations due to an AE or any other cause.

Altogether 17 treatment-emergent AEs were reported by 6 subjects (4 females and 2 males) during the study (Table S1). Three of 17 AEs in 3 subjects were moderate, while the other AEs were mild. The most frequently reported treatment-emergent AEs during the study were itching (5 events in 3 subjects), and headache (3 events in 2 subjects); all other AEs were reported by single subjects only. Six of the 17 treatment-emergent AEs were reported as treatment-related AEs; all reported as mild and skin-related, as detailed below.

Table S1. *Summary of adverse events (AEs) during the study*

	Males ($n=7$) Events (subjects)	Females ($n=9$) Events (subjects)	Total ($n=16$)
All AEs	5 (2)	12 (4)	17 (6)
Treatment- related AEs	0 (0)	6 (3)	6 (3)
Skin-related AEs	0 (0)	9 (4)	9 (4)
Other AEs	5 (2)	3 (2)	8 (4)

Nine out of 17 treatment-emergent AEs were skin-related, reported by 4 subjects (all females). Seven of these skin-related AEs occurred on forearm on the application area. Six skin-related AEs in 3 subjects occurred on the volar forearm on the control vehicle application area, and were all reported as treatment-related AEs. These were itching (5 events in 3 subjects: on day 1; on days 3–5; 2 events on day 5; on days 7–8) and formation of papules (1 event on days 3–5). They all were regarded to be related to administration of the vehicle, Aqualan® emulsion cream. There were no skin-related AEs that were reported to have a positive causal relationship to cis-UCA treatment.

The 2 skin-related AEs (2 events in 2 subjects) occurring outside the application areas were radiating pain in wrist area probably due to blood sampling, and dry eczema below right lower eyelid probably due to the use of a new eye shadow.

Other AEs (i.e., not skin-related; 8 events in 4 subjects) were as follows: ear pain and otitis media (same subject), exacerbating headache, stomach ache and vomiting (same subject, same day); mild intermittent headache and mild headache (same subject); and muscular pain after bicycle injury. They all were reported as not having a positive causal relationship to the study treatments.

Measurement of treatment compliance

The morning doses were administered by the study personnel and the compliance was therefore 100% for those doses. According to subject diaries, all evening doses were administered by the subjects. In 2 cases, the administrations were performed out of acceptable time limits; in one case 20 min too late and in another case, one min too early.

Statistical methods and determination of sample size

Descriptive statistics by visit (study day) and time point were provided to summarise the study results, including changes from baseline where applicable. Summary statistics included the number of subjects, mean, standard deviation, standard error, minimum, median and maximum for continuous variables, and frequencies and percentages for categorical variables. No imputations were done for missing observations. No interim analyses were performed. No formal hypotheses were tested in this explorative study and, as a consequence, no multiple testing adjustments were performed. A two-sided p-value of < 0.05 was considered statistically significant.

The number of subjects planned to be included in this study was based on clinical considerations. A formal sample size calculation was therefore not performed. Sixteen healthy volunteers (7 males and 9 females) were included in the study as planned in the study protocol (6–10 males and 6–10 females).

STUDY 3: Double-blind, vehicle-controlled, two-phase, single centre Phase I/IIa study with topical twice daily doses of 5% cis-urocanic acid to investigate pharmacokinetics, safety, tolerability and efficacy for up to 28 days in subjects with mild to moderate atopic dermatitis

Inclusion criteria

Identical to criteria used in Study 2, except for the following points;

1. Subjects with chronic mild to moderate atopic dermatitis with the following diagnostic features:
   - History within 12 months of itching dermatitis in one or several of localizations typical of atopic dermatitis (antecubital/cubital fossae; face/neck/upper trunk; volar aspects upper extremities/thighs).
   - Objective signs of mild or moderate eczema or dry skin in one or several of above mentioned locations
2. No current treatment with active medication for atopic dermatitis.
3. Symmetric presentation of atopic dermatitis in the volar forearms to allow randomisation of the treatments.
4. Good general health ascertained by medical history, physical examination, ECG recording and laboratory determinations, showing no signs of clinically significant findings, except chronic atopic dermatitis.

Exclusion criteria

Identical to criteria used in Study 2, except for the following points;

Subjects were not included if they fulfilled one or several of the following:

1. History of other significant skin disease (e.g. any skin disease requiring hospitalisation), or skin manifesta-

tions of allergic illness or other dermatologic condition, except chronic mild to moderate atopic dermatitis, that would interfere with the trial assessments or compromise the subject's safety according to the opinion of the investigator.

2. Present symptoms of other skin diseases, except chronic atopic dermatitis, that could disturb the study assessment and evaluation of the skin, according to the opinion of the investigator.
3. Current use of any active systemic medication (i.e., oral, subcutaneous, intravenous) for chronic atopic dermatitis within one month (30 days).
4. Current use of active topical medication in the investigational area for chronic atopic dermatitis within one month (30 days).
5. Asymmetric presentation or only single lesion of atopic dermatitis on volar forearms.

Instructions concerning lifestyle and concomitant medications

At the screening visits, the subjects were advised to comply with the following instructions (as provided in the original protocol). These instructions were applicable for PK phase (instructions applicable during Extension phase are provided below):

1. The subjects will have to refrain from strenuous physical exercise for 48 h before the 1st screening visit (including laboratory assessments), and end-of study visit, and before the treatment administrations on days 1 and day 10, and until the end of blood sampling after day 10 (i.e., day 11 morning).
2. The subjects will have to refrain from the use of prescription drugs within 14 days prior to dosing or over the counter medication within 7 days prior to dosing (including herbal remedies, trace elements, vitamins, dietary supplements) and during the study. Paracetamol may be taken for occasional pain. In addition, antihistamines and use of non-steroidal nasal sprays and eye drops are allowed for allergic symptoms.
3. The application area should remain intact and it should not be touched e.g. by a finger for at least 2 h after each application.
4. The subjects will have to refrain from taking showers more than twice daily during the study medication until the end of blood sampling on day 10 (exceptions in point 10).
5. The subjects should take their morning shower (if any) before their visit to the Phase I unit. Likewise, they should take their evening shower (if any) before the application of the evening dose (exceptions in point 10).
6. The subjects will have to refrain from sauna bath during the study medication until the end of blood sampling after day 10.
7. The subjects will have to refrain from consuming alcohol products for 48 h before the screening visits, before the day 1 and 10 visit, and until the end of blood sampling and urine collection after day 10, and before the end-of-study visit.
8. The subjects must fast for at least 10 h before each safety laboratory determination (at screening and at end-of-study visit) and before the 1st treatment administration on days 1 and 10.
9. The subjects are allowed to have only meals and drinks served by study personnel at the study site while confined for PK assessment on study day 10. Water is allowed from 4 h on study day 10 *ad libitum*. No restrictions in water consumption are needed on other study days.
10. Restrictions concerning the treatment areas for PK assessments on study day 10: The subjects are not allowed to touch the treatment areas with fingers, clothes or by other means at least for 2 h or even longer until all of the emulsion cream is absorbed in the skin. The subjects are not allowed to let the treatment area to get wet for approximately 12 h after the administration. The treatment areas must not be scrubbed or scratched during the 12 h after the treatment. In addition, shower is not allowed during 12 h after the administration.
11. The subjects must wash their hands before and after the application of the study treatments with mild soap and water, carefully avoiding that the treatment area will not get wet.
12. The subjects are to report all AEs and concomitant treatments to the study personnel. Minor AEs may be reported at visits and significant as soon as possible.
13. At home study subjects are to fill the diary (AEs, concomitant medications, other deviations, evening dose application) starting from the screening visit until the end-of study visit.
14. The subjects are to collect the urine in containers from day 10 to day 13 (until 72 h after the dose on day 10) and to bring the containers to the Phase I unit as requested.
15. The subjects are instructed not to participate in another clinical drug study or donate blood within 60 days (males) or 90 days (females) after the last treatment dose of this study.
16. The subjects are instructed to use adequate contraceptive measures during the study and at least 30 days after the last cis-UCA dose. (See inclusion criteria 8).

The subjects were advised to comply with the following instructions (as provided in the original protocol) during Extension phase. The subjects were advised to comply with the instructions listed above also during Extension phase, if applicable.

1. The subjects will have to refrain from taking showers more than twice daily during the study medication
2. The subjects should take their morning shower (if any) before the application of the morning dose. Likewise, they should take their evening shower (if any) before the application of the evening dose.
3. The subjects will have to refrain from use of sauna bath for 48 h before the visit days during the extension period, and they should refrain from excessive use of sauna bath (more than twice per week) during the study medication.
4. The subjects must fast for at least 10 h before safety laboratory determination (at end-of-study visit).

5. The subjects will have to refrain from strenuous physical exercise for 48 h before the end-of study visit.
6. The application area should remain intact and it should not be touched, e.g. by a finger, for at least 2 h after the application.
7. The subjects must wash their hands before and after the application of the study treatments with mild soap and water, carefully avoiding that the treatment area will get wet.
8. The subjects are to report all AEs and concomitant treatments to the study personnel. Minor AEs may be reported at visits and significant as soon as possible.
9. At home study subjects are to fill the diary (AEs, concomitant medications, other deviations, treatment dose applications) starting from the screening visit until the end-of study visit.
10. The subjects are instructed not to participate in another clinical drug study or donate blood within 60 days after the last treatment dose of this study.
11. The subjects are instructed to use adequate contraceptive measures during the study and at least 30 days after the last cis-UCA dose.

Disposition of subjects

Of the 42 screened subjects, 14 were included in the study. The reasons for exclusions for the 28 subjects were as follows: too mild atopic dermatitis on volar forearms (21 subjects), too strong atopic dermatitis, high heart rate, use of non-allowed concomitant medication, decreased blood haemoglobin value, history of recent epidemic nephropathy, and susceptibility to severe allergic reactions (2 subjects). The subjects were instructed to further examinations, if clinically judged.

One subject withdrew her consent after randomisation, as her atopic eczema had worsened markedly and she wanted to start corticosteroid treatment. The pharmacokinetic phase was started and completed by 13 subjects. Another subject expressed unwillingness without giving a reason to continue to the extension phase which was completed by 12 subjects.

The 1st subject was screened on 14 October 2008. The 1st dose was administered on 18 November 2008 and the last dose on 19 May 2009. The last subject completed the study on 28 May 2009.

Adverse events

Study treatments were well tolerated and safe. There were no serious or other significant AEs during the medication period. There were no discontinuations due to an AE.

Altogether 30 AEs were reported by 10 subjects (9 events in 3 males and 21 events in 7 females); 22 events in 7 subjects were treatment-emergent AEs (9 events in 3 males, 13 events in 4 females). Altogether 13 skin-related AEs (5 cases in 2 males and 8 cases in 2 females) were reported, of which 12 were treatment-emergent. The most frequent treatment-emergent skin-related AEs were pruritus (5 events in 1 subject) and worsening of atopic dermatitis (2 events in 2 subjects); all other events were reported by single subjects only. The most common 'other' treatment-emergent AEs by system organ class were nervous system disorders (3 occasions of headache in 2 subjects) and infections (2 occasions of upper respiratory tract infections and 1 common cold); all other events in this category were single reports.

Seven out of the 22 treatment-emergent AEs were considered moderate (4 events of itching in 2 subjects, dyspnoea, worsening of atopic dermatitis, and bursitis), whereas the other 15 were considered mild.

There were 6 treatment-related AEs in 1 female subject; 4 occasions (3 moderate and 1 mild) of pruritus on the skin including investigational areas on both treatment sides 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.

In safety laboratory assessments at the end of the study, there was one ALT elevation (ALT 78 U/l), which was considered a clinically relevant abnormality and reported as an AE (not related to the study medications). No clinically significant findings were found in vital signs or ECG of any subject at the end of the study.

Measurement of treatment compliance

There were 6 cases of missing administrations in 4 subjects. Study medication administration time deviations were recorded in 48 cases in 10 subjects. In 25 cases the administration was performed 3–115 min later and in 1 case 34 min earlier than the acceptable time limit. The time between morning and evening administrations was too long in 14 cases and too short in 8 cases.

Statistical methods and determination of sample size

Descriptive statistics by treatment group and study day were provided to summarise the study results for the intent-to-treat dataset. Summary statistics included the number of subjects, mean, standard deviation, standard error, minimum, median and maximum for continuous variables, and frequencies and percentages for categorical variables. For safety and tolerability, non-parametric Wilcoxon signed rank test was used to analyse the VSS scores. For efficacy, repeated measurements analysis of covariance (RM ANCOVA) model over the 2 treatment phases (i.e., study days 5, 10, 21, and 28) was used to analyse the decreases from baseline (study day 1) in skin erythema, TEWL, and EASI scores. Non-parametric Wilcoxon signed rank test was used to analyse the PGA scores. No formal hypotheses were tested in this explorative study and, as a consequence, no multiple testing adjustments were performed. A two-sided p-value of < 0.05 was considered indicative of statistical significance. No interim analyses were performed and no imputations were done for missing observations.

The number of subjects planned to be included in this study was based on clinical considerations. A formal sample size calculation was therefore not performed. Thirteen adult subjects with mild to moderate atopic dermatitis (5 males and 8 females) were included in the study, almost as planned in the study protocol (6–10 males and 6–10 females). All the 13 subjects were eligible for the Extension phase, and 12 subjects continued to it.