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ORIGINAL ARTICLE

Topically applied novel TRPV1 receptor antagonist, ACD440 Gel, reduces evoked pain in healthy volunteers, a randomized, double-blind, placebo-controlled, crossover study

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Abstract

Background: The TRPV1 receptor is a key molecule in pain generation. Previous development of oral TRPV1-antagonists was halted due to systemic heat insensitivity and body temperature alterations. The present Phase 1b study investigated the efficacy, safety and plasma exposure of a topically administered TRPV1-antagonist (ACD440 Gel) in healthy subjects.

Methods: The study comprised two parts.

In part 1, 24 healthy subjects were included in this randomized double-blind, placebo-controlled, crossover trial. ACD440 Gel or Placebo was applied once daily and wiped off after 1 h, for 5 consecutive days. Assessments were done in normal skin, skin optimized for penetration (by stripping and occlusive gel application) and UVB-irradiated skin. Pain induced by thermo-nociceptive CO_2 laser impulses generated laser-evoked potentials (LEPs), with readouts of peak-to-peak (PtP) amplitude in vertex-EEG and pain assessments by VAS (0–100). Endpoints include effects at 1 hour post-dose, AUC(Days 1–5) and $AUC_{(0–24,\ Day\ 4)}$. In UVB-irradiated skin, also pain on pinprick and skin redness were assessed.

Part 2 explored the plasma pharmacokinetics of ACD440.

Results: ACD440 Gel reduced LEP PtP amplitude and VAS pain, $p < 0.001$, in all skin conditions, versus placebo. In UVB-irradiated skin, pinprick pain was also reduced, $p = 0.047$. Effects were significant after 1 h, maintaining for at least 9 h. There were no adverse events or drug-induced erythema. Plasma exposures of ACD440 were too low to establish an elimination half-life of ACD400.

Conclusions: Topical ACD440 Gel demonstrated a significant analgesic effect on LEP, VAS score and pinprick pain, with low systemic exposures, supporting further clinical development.

Significance: This study demonstrates that the topical administration of a TRPV1-antagonist, ACD440 Gel, has potential as a new treatment for painful

conditions affecting the skin, such as chronic peripheral neuropathic pain, without any local or systemic side effects.

1 | INTRODUCTION

Neuropathic pain is caused by lesions or diseases of the somatosensory nervous system, where pain intensity of clinical relevance affects 7%–10% of the general population (IASP, 2011; van Hecke et al., 2014). Many underlying diseases can cause chronic peripheral neuropathic pain, for example, diabetic polyneuropathy, chemotherapy, traumatic or perioperative nerve injury. These pain conditions have common phenotypic features related to disturbed small fibre function, well described by Baron, Vollert and others (Baron et al., 2017; Vollert et al., 2016). According to these studies, a substantial proportion of patients have some degree of sensory hypersensitivity. The capsaicin (vanilloid) receptor, TRPV1, is a nonselective cation channel expressed by a subset of small diameter unmyelinated (C) and myelinated (Aδ) nociceptors (Basbaum et al., 2009) as well as by non-nociceptive primary afferents and non-neural cells. Clinical data by Petersen have demonstrated increased expression of TRPV1 receptors in painful skin of postherpetic neuralgia (Petersen et al., 2002). Currently available treatments still leave many patients without sufficient pain relief. Therefore, there is a strong need to identify analgesics for the treatment of chronic peripheral neuropathic pain with both improved efficacy and better benefit–risk ratio than current treatments. Thus, a topical treatment with good efficacy, local tolerability and low systemic exposure would be highly advantageous. Current topical products, such as lidocaine patches and 8% capsaicin patches (Qutenza®), are considered second- or third-line treatments for peripheral neuropathic pain, due to insufficient evidence of effectiveness and/or treatment-related pain (Finnerup et al., 2015). A potent topical compound acting as an antagonist of the TRPV1 channels would be a novel non-opioid approach to obtain analgesic effect without associated side effects of existing systemic therapies.

Several TRPV1 receptor antagonists were in clinical development during the beginning of this century, intended as orally administered pain treatment. The development of these candidates was stopped due to target-related systemic effects, that is, effective blocking of heat sensations, leading to an increased risk of adverse effects due to burn or scalding, or drug-induced hyperthermia (Arendt-Nielsen et al., 2016; Manitpisitkul et al., 2016, 2017; Rowbotham et al., 2011). The hyperthermic effect of oral treatment is considered due to the inhibition of heat sensation in the gut (Yue et al., 2022), supporting the development for topical use of TRPV1 antagonists, avoiding dose limiting target-related effects.

ACD440 is a potent and selective TRPV1 antagonist under development by AlzeCure Pharma AB for the treatment of peripheral neuropathic pain (Sjögren et al., 2018, 2019). Local blocking of the TRPV1 receptor may circumvent the target-related adverse events experienced after oral dosing. Therefore, a topical gel formulation of ACD440 has been developed as a novel treatment option for peripheral neuropathic pain.

The current study aimed to demonstrate proof of mechanism of the dermal gel formulation of ACD440, by exposing healthy subjects to laser-induced pain on normal skin, on skin optimized for penetration and on ultra-violet-B-irradiation (UVB) induced skin erythema. We hypothesized that ACD440 Gel would significantly reduce the nociceptive pain induced by radiant-heat laser stimulation and UVB-induced inflamed skin compared to placebo gel. Furthermore, safety and tolerability as well as plasma exposure to ACD440 were explored.

2 | METHODS

This was a prospective, double-blind, randomized, placebo-controlled study of pharmacodynamic (PD) efficacy, safety and pharmacokinetics (PK) of the TRPV1 antagonist ACD440 Gel. The study was conducted at the CRO HPR Dr Schaffler GmbH (Human Pharmacodynamic Research), Munich, Germany, in accordance with the Declaration of Helsinki as adopted by the World Medical Association in 1964, and lastly amended in 2013. The study was conducted in accordance with Good Clinical Practice, after regulatory approval by the BfArM (EudraCT 2020-003597-49) and ethics approval by the Bavarian regional committee. The study was preregistered on www.clinicaltrials.gov (NCT04704232).

2.1 | Study design and objectives

The study was conducted in two parts:

Part 1 (PD) was a prospective, double-blind, randomized, placebo-controlled pharmacodynamic study evaluating the analgesic efficacy, safety and plasma exposure of ACD440 Gel 14 mg/g (here for control reasons only) when applied to normal skin, skin optimized for

drug penetration and skin exposed to UVB irradiation in healthy subjects (Figure 1).

Part 2 (PK), was a randomized, double-blind, placebo controlled study aimed to explore the systemic exposure of ACD440 after application of ACD440 Gel dosed with 14 mg/g to a clinically relevant skin surface area. Part 2 (full PK) included eight of the subjects having already participated in Part 1.

In Parts 1 and 2 of the study, study treatments consisted of the TRPV1-receptor antagonist ACD440 Gel or placebo gel, administered at a volume of 0.05 mL/cm^2.

The study objectives were:

For Part 1 (PD):

a. To compare the effect of ACD440 Gel to placebo if applied on skin optimized for drug penetration (skin stripping, occlusive gel application) using vertex EEG LEPs and subjective pain rating, using an electronic 100 mm VAS.
b. To compare the effect of ACD440 Gel to placebo in reducing the overall peak-to-peak (PtP) amplitudes of LEPs and VAS on normal skin.
c. To compare the effect of ACD440 Gel to placebo in reducing UVB irradiation induced hyperalgesia using LEPs, VAS and weighted needle threshold (WNT) to define (mechanical) pinprick hyperalgesia.
d. To compare the effect of ACD440 Gel to placebo in reducing UVB irradiation induced erythema (inflammation) using skin reflection spectrometry (SRS).
e. To evaluate systemic safety and tolerability of a topical application of ACD440 Gel.
f. To evaluate local tolerability of ACD440 Gel on normal skin, skin with an impaired barrier and inflamed (UVB exposed) skin.

For Part 2 (PK):

g. To evaluate plasma exposure of ACD440 after topical application of ACD440 Gel 14 mg/g (PK sub-study).

2.2 | Subjects

Twenty-six healthy male and female adult subjects of aged 18–64 years were screened, out of which 24 were found eligible and included in this double-blind, randomized, placebo-controlled crossover trial after providing their verbal and written informed consent.

Additional important inclusion criteria, apart from being in general good health, included subjects being of Fitzpatrick skin type II or III (covering 90% of the European population—due to the applied UVB irradiation dose), be willing to avoid sun exposure, tanning lamps/studios and use of any

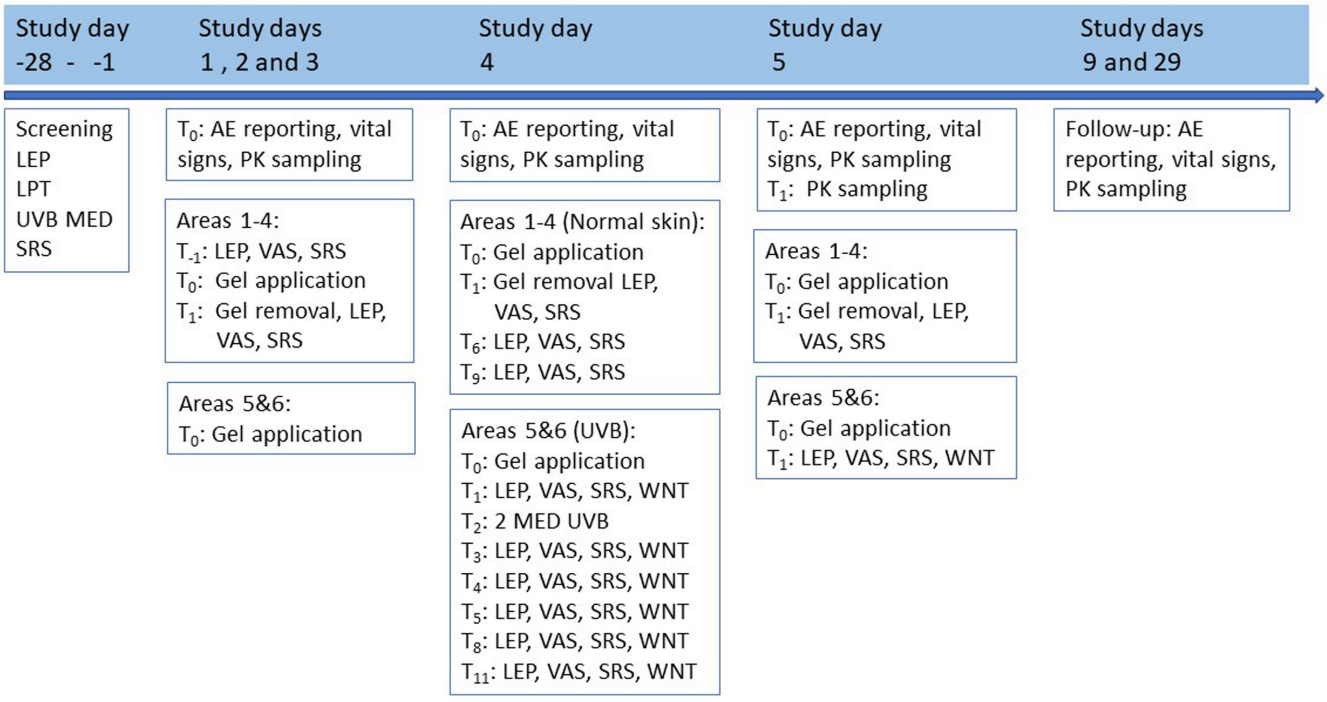


FIGURE 1 Flowchart of the study procedures and assessments during Part 1 of the study. AE, adverse event reporting; LEP, laser-evoked potentials; LPT, laser-evoked pain threshold; PK, collection of blood samples for plasma concentration of ACD440; SRS, skin reflection spectrometry; T_x, time point (in hours) for the specified baseline assessments for each experimental day, with T_0 depicting start of first set of study procedures, with gel application to areas 1–6 being done as the last activity from which timing started; VAS, visual analogue scale; UVB MED, assessment of the UVB minimal erythema dose; WNT, Weighted Needle Threshold.

topical or cosmetic products on the test areas throughout the study. Furthermore, subjects needed to show distinct/clean amplitude responses in laser-evoked potentials (LEPs) from 12 artefact-free vertex-EEG-segments after phase-free filtering, averaging and online evaluation of peak-to-peak (PtP, N2 plus P2 components' maximum) amplitudes as target variable (Figure 2a–c).

Exclusionary criteria included having a positive Covid-19 PCR test, known hypersensitivity to ingredients of the test products, and having tattoos, photosensitivity or skin diseases in the intended test areas. Ongoing pain conditions that could interfere with study assessments were also exclusionary. Participating subjects were not allowed to take any analgesic therapy or other drugs that might alter pain perception, including centrally active drugs, nor taking any systemic or topical drugs that could affect responses to UVB irradiation or could give rise to dermatological adverse reactions.

Active study treatment consisted of the TRPV1-receptor antagonist ACD440 Gel 14 mg/g, 0.05 mL/cm^2. In study part 1, 24 subjects were administered 1 mL of study drug topically once daily, evenly spread onto 20 cm^2 of each of the six skin areas to be investigated (Figure 3). Placebo treatment consisted of the same excipients as the active drug. Part 2 of the study, a PK sub-study, included eight healthy subjects having already participated in the main study. On Day 1, 3.33 mL of ACD440 Gel was applied on 66.6 cm^2 skin area (0.05 mL/cm^2) once. On Day 8, 10 mL of ACD440 Gel was applied once onto 200 cm^2 skin area (0.05 mL/cm^2).

2.3 | Study-specific methodologies

2.3.1 | Laser-evoked potentials (LEP)

For LEP methodology and validation, see also Schaffler et al. (2012) and Schaffler et al. (2017).

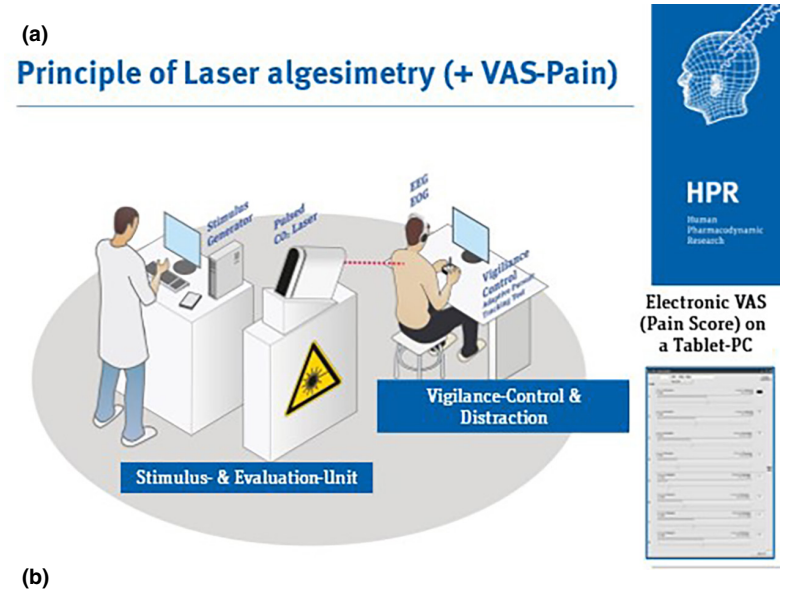
A pulsed CO_2 laser (SYNRAD infrared gas laser model E48-1/10 W, SYNRAD Inc, North Bothell, WA, US) with a spectral emission in the far-infrared, 10,200–10,600 nm, a direct beam diameter 3.5 mm and a single stimulus duration of 80 ms was used for sensory stimulation. Stimulus presentation was done in random intervals of 4–8 s, and by stepwise automatic changes to another skin location after each shot by about 3 mm.

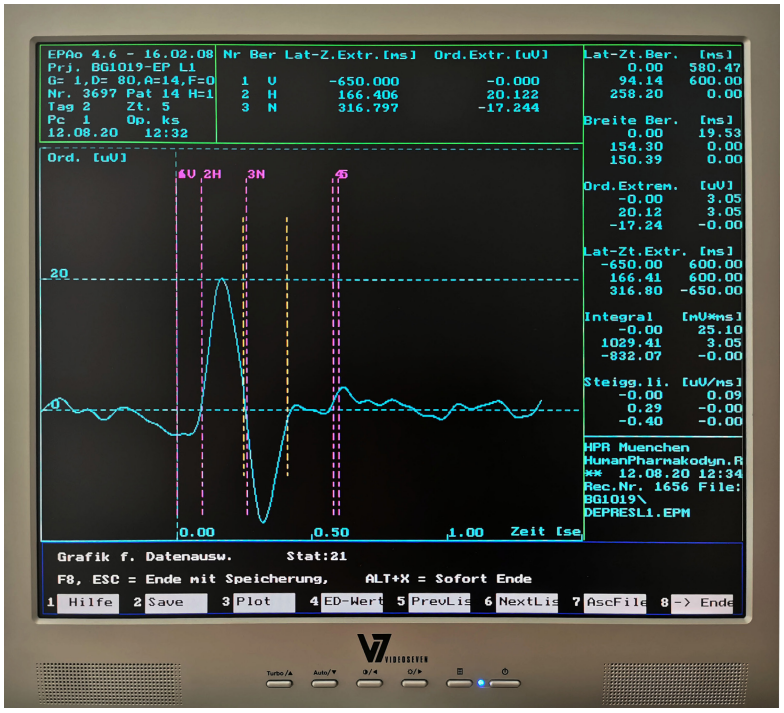
The major advantage of this technique is that heat-sensitive ion channels of thermo-nociceptors (e.g. TRPV1) of Aδ (thinly myelinated) and C-fibre (non-myelinated) type are opened. Those channels are selectively stimulated by means of the CO_2 laser beam with a low depth of penetration (at free nerve terminal level) due to its wavelength with a maximum absorption in water.

LEP signals (Figure 2b,c) were automatically obtained from 10 to 20 EEG positioning system of the electrodes at vertex versus right mastoid and at canthus versus nasion for EOG. Both were used for automatic artefact rejection (of eye blinks, facial electromyographic influences and of abnormal EEG baseline drifts). Surveillance of artefacts was done online for a period of 650 ms before random stimulation—resulting in a suppression of the initiation of the respective stimulus and continued until 600 ms after each stimulation—then resulting in a complete rejection of this single segment for further evaluation. Registration was done via programmable (differential) bio-amplifiers with an analogue filter setting of 0.15–30 Hz and by online real-time computer averaging of 12 artefact free sections. The resulting biosignal additionally passed a digital Gaussian phase-free filter on the basis of a Fast Fourier Transform (FFT), sampled with a digitization rate of 512 Hz.

The resulting two main evoked potential components with a maximum amplitude (for LEP curve analysis, see also Figure 2b,c) were evaluated regarding their complex PtP amplitude, as well as the single N2 component (with a latency at about 150–160 ms, Schaffler et al., 2017), primarily reflecting peripheral (i.e. stimulus-related) effects, and the P2 component (with a latency at approximately 260–280 ms, Schaffler et al., 2017), primarily reflecting central (i.e. processing and cognitive) effects.

During stimulation, the subjects were sitting on a chair, with their arms resting on a table in front of them and their head fixed in an ophthalmologic forehead–chin rest for positioning and relaxation of neck muscles to avoid myogenic artefacts. Since alterations in vigilance have an impact on EP amplitudes, there was a need for vigilance control during LEP assessment. This was accomplished by means of loading the subjects with a pursuit tracking task on a computer screen, which was performed for the entire period of each LEP recording session. Subjects were exposed to 'white noise' during the entire assessment via earphones (with a sound pressure of about 85dBA) to avoid influences of external disturbing noise (to raise and stabilize vigilance) and to distract subjects from pain stimulation and pain sensation expectancy. Due to automatic artefact detection, Gaussian phase-free filtering and averaging the evaluation of stimulus-triggered EEG data can be conducted online during registration. The LEP PtP amplitudes were defined as the main target variables for the investigation of analgesic effects of ACD440 Gel versus placebo gel. Analgesic effects of active treatments were generally expected to result in a reduction of the N2 and/or P2 amplitudes.





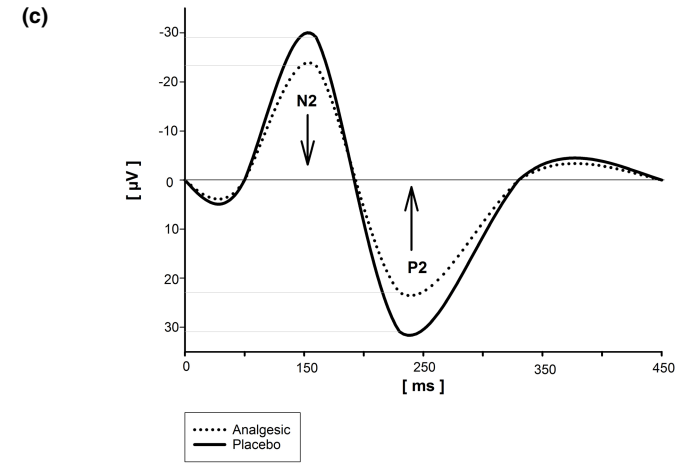


FIGURE 2 (a) Principle of experimental laser-evoked potential (LEP) study set-up. (b) Original LEP signal outcome of a single stimulation session—mean out of 12 laser shots with online determination of latency and amplitudes. (c) Illustration of the N2 P2 peak-to-peak (PtP) amplitude principle with theoretical analgesic drug effect versus placebo (indicated by amplitude suppression).

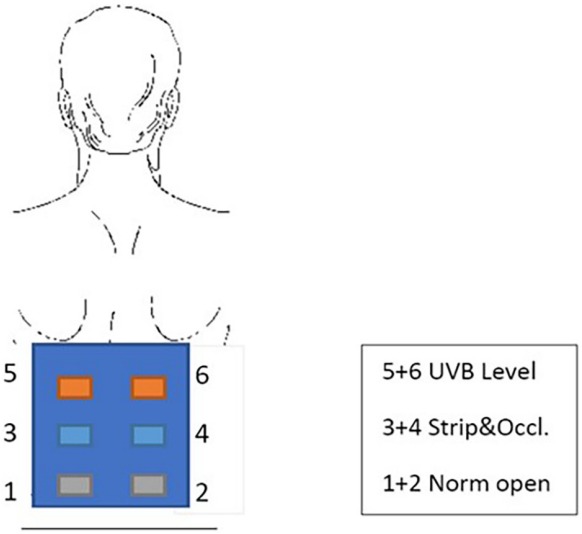


FIGURE 3 Localization of treatment areas 1–6. Within each pair of areas, these were randomized to receive either ACD440 or placebo. The skin was tested under three different conditions: normal non-occluded (Norm open) on areas 1 and 2, stripped skin with occlusive plastic film (Strip & Occl.) on areas 3 and 4 and UVB-irradiated skin (UVB Level) on areas 5 and 6.

2.3.2 | Laser-evoked pain thresholds

At screening, the individual (thermo-nociceptive) CO_2 laser pain threshold (LPT)—induced by the Synrad infrared gas laser model (see above)—was determined by the application of slowly increasing laser beam intensities to the normal skin of each participant until they felt a distinct pinprick sensation. The intensity was finally adjusted to 50% higher than this threshold. Once determined at screening in normal skin, the intensity of the laser stimuli and also of the applied twofold minimal erythema dose (MED) of UVB irradiation (determination see below) was both kept constant over the entire study period for each individual.

2.3.3 | Pain intensity assessments

Post-laser stimulus pain intensity (VAS pain) was assessed by using an electronic visual analogue scale (VAS) on a tablet PC, which allows a 0–100 mm or % definition between no pain and maximum pain. The measurement was done after each laser session, summarizing retrospectively the overall impression of the 'painfulness' of the whole session.

2.3.4 | UVB-irradiation exposure to the skin

The UVB model is a well-validated model for investigating the effect of anti-inflammatory and analgesic compounds with a primary peripheral mode of action (Schaffler et al., 2012, 2017). Effects on pain perception (nociception) and hyperalgesia due to inflammation by UVB irradiation were measured by radiant-heat laser algesimetry (Schaffler et al., 2012, 2017). The UVB radiation (280–320 nm of invisible spectral range with a narrow band emission at 311 nm) is known to distinctly enhance redness (erythema) of the skin.

At screening, UVB was applied in six ascending doses once to six small areas ($1 \times 1\,cm^2$ each $= 6\,cm^2$) of the skin on the subjects' backs, in order to determine the minimal erythema dose (MED). The UVB irradiation was provided using a Dermalight® 80 with a narrow-band UVB emission at 311 nm (invisible range), which contains Philips TL 9 W/01 UVB tubes. The first skin area with a regular discernible and well-defined square erythema (after a development time over 8–10 h at least) was recorded as the individual MED.

In the morning of Day 4, the twofold individual MED was applied using the Dermalight 180® narrow-band UVB source (with a narrow-band emission, as above, A.L.T. Lichttherapietechnik GmbH, Zörbig, Germany). The UVB exposure was performed on two skin areas of the back (2 areas of $5 \times 4\,cm = 20\,cm^2$ each at skin position 5 and 6, Figure 3) to produce a homogenous area of skin erythema and hyperalgesia that was large enough to perform several repeated laser sessions. Once determined at screening on normal skin, the intensity of the UVB dose and laser stimuli the for each individual subject was kept constant over the entire study period.

2.3.5 | Weighted needle (WNT) assessment of mechanical/mechanosensory hyperalgesia

The WNT assessment of mechanical hyperalgesia was only performed in the primary UVB-exposed area by fixed weight steps at the same session time points as done with LEPs+VAS pain (always after these sessions) (Figure 1). Skin contact was made by a rounded/blunt needle tip placed on the skin with a defined weight (in mN). The WNT set was supplied by Institute of Physiology and Pathophysiology of University Erlangen-Nuremberg in a calibrated status of 19 steps in contrast to quantitative sensory testing (QST) with only seven steps in Rolke et al. (2006) (see also 'Deutscher Forschungsverbund Neuropathischer Schmerz'—DFNS). The weight range from 64 to 512 mN was used in this study. Upon stimulation, subjects reported at which weight they felt any pain (threshold value) or only pressure in the respective testing area. In WNT, an increase in threshold values is a positive analgesic effect (in contrast to LEP amplitudes and to VAS pain score with a decrease as outcome for a positive drug effect).

2.3.6 | Skin reflection spectrometry (SRS)

Quantitative measurement on UVB-exposed skin areas (regarding erythema intensity/flare) or on normal skin 'irritation areas' (AE check) was done by a Chroma Meter CR-400 (Konica-Minolta Optics, Inc., Munich), looking for spectral shifts, respectively, here for changes of the colorimetric parameters according to the CIE-Lab system (CIE = Commission Internationale l'Éclairage, Lab = Colour dimensions for SRS, L = luminescence, a = red/green dimension, b = blue/yellow dimension). 'Cold' polychromatic light was guided to the skin and to a photomultiplier. Output variables were colorimetric parameters according to the CIE Lab system; in this case, the a-value ('redness'), a parameter without any dimension. An anti-inflammatory effect of a drug results in a lower a-value of the Lab measurement system (= less redness). The a-value was determined at the following time points: prior to any ACD440 Gel/placebo administration, and at 1 h after application, after removal of the gel, as described in Figure 1.

SRS measurements were done to compare the effects of ACD440 Gel to placebo gel in influencing UVB-induced erythema (inflammation) and to assess any skin irritation in normal skin that would be caused by the active ingredient of the ACD440 Gel compared to placebo gel. The latter was used as a safety assessment.

2.4 | Study procedures

2.4.1 | Study treatment, randomization and blinding

In both Parts 1 and 2 of the study, the independent study statistician provided blinded randomization lists to an unblinded member of the study team (a pharmacist) who was not involved in the study course or any evaluation of outcome criteria. The pharmacist prepared the specific individual dosages from bulk material of ACD440 Gel and placebo gel in a separate area not accessible to study staff conducting or assessing efficacy or safety of the study treatments. The application of the study medication (ACD440 Gel or placebo gel) was done by a blinded member of the study personnel. The randomization lists were kept strictly confidential, not accessible to blinded members of the study team. Unblinding of the randomization code took only place after official database closure.

2.4.2 | Study part 1, Pharmacodynamic (PD), main study part

Healthy subjects were screened for eligibility from Day −28 to Day −2 before first treatment. Prior to testing the LEPs from skin, a training session was performed during the screening period to introduce subjects to the testing and rating procedures (Figure 1).

On Day 1, enrolled subjects were irradiated with six different increasing dosages of UVB radiation to establish their individual minimal erythema dose (MED = area of UVB exposure which produced the first clearly discernible erythema). On day 2, the read-out to determine this MED was done as per definition.

Six drug treatment areas, each with a size of 20 cm^2 was defined at Day 1, all in the lumbar and dorsal thoracic area (as to be seen in Figure 3).

Areas 1 and 2 were defined for treatment on normal skin. Areas 3 and 4 were used to examine optimized application conditions using skin stripping to remove parts of the stratum corneum using a standardized stripping protocol (10 times stripping with adhesive tape per each area [3 and 4] after preceding soft mechanical skin cleaning), followed by the gel administration and then by the application of an occlusive bandage. Areas 5 and 6 were reserved for the exposure to UVB irradiation. Areas 1–6 were pairwise randomized to treatment with either ACD440 Gel or placebo (three areas each). Treatment was performed once daily, in the morning, for 5 consecutive days. Study medication was left on the skin for 1 h, and then wiped off in order to enable study assessments.

Study procedures for normal skin and optimized skin were performed on days 1–5 on skin areas 1–4 (Figure 1). On study days 1, 2, 3, 4 and 5, areas 1–4 were tested for LEP-amplitudes and pain intensity (VAS 0–100) before and at 1 h after application of 1 mL of study medication (ACD440 Gel 14 mg/g (0.05 mL/cm^2) or placebo). On day 4, LEPs and VAS (0–100) were evaluated also at 6 and 9 h after application of study medication.

Study procedures for the UVB irradiation on areas 5–6 were performed on days 4 and 5. At the 2 h post-dose time point on Day 4, skin areas 5 and 6 were UVB irradiated with 2 MED. LEPs, VAS (0–100), erythema and pin prick hyperalgesia (WNT) were evaluated before and 1, 2, 3, 6 and 9 h after UVB irradiation. At the same time points, anti-inflammatory effects on the areas of UVB irradiation were evaluated by SRS with the a-value being the measure of 'redness'. On day 5 morning, study medication was applied once. One hour after application and 24 h after UVB irradiation, LEPs and VAS (0–100), erythema (SRS) and pin prick hyperalgesia (WNT) were evaluated on areas 5 and 6.

Blood sampling for plasma exposure, study part 1: On days 1 and 2, blood was collected immediately before administration of study medication. On day 5, blood was collected at 1 h after administration of study medication. Additional blood samples were collected at the follow-up visits on Days 9 and 29.

Safety assessments included a physical examination, vital signs (pulse rate, blood pressure, auricular body temperature) and unsolicited reporting of adverse events. In addition, SRS was performed on normal skin areas subjected to study medication (areas 1–4) but not to UVB irradiation for this purpose, to obtain objective evaluation of any induced skin erythema, which would then have been considered an adverse event.

2.4.3 | Part 2, pharmacokinetic (PK) substudy

Eight subjects who had finished the first part of the study were included in the PK substudy to be started minimum 14 days after end of Part 1 (PK Day 1). Those PK subjects (PK population) randomly received either ACD440 Gel ($N=6$) or placebo ($N=2$). This part was conducted in two sessions. At the first session, subjects were treated with 0.05 mL/cm^2 of ACD440 Gel ($N=6$) or placebo ($N=2$) administered at an area of 66.6 cm^2. At the second session, occurring 7 days later, subjects were administered 10 mL of study medication (0.05 mL/cm^2) to a treatment area of 200 cm^2. On both occasions, blood samples for PK analysis were collected before and at 0, 1, 2, 3, 6, 9 and 24 h after application. Safety was assessed at the same time points. A final PK follow-up visit was done on PK Day 15, including a final PK sample and a safety assessment. Plasma was separated from blood and frozen at −70°C. Bioanalysis of ACD440 was performed by LC-MS/MS (Charles River Laboratories, UK).

2.5 | Statistical considerations

The power analysis for the pairwise comparison was based on a linear mixed regression model for the area under the curve (AUC) parameter of the PtP amplitudes of LEPs. AUC as instrument for evaluation was chosen due to the cumulative drug administration over 5 days, as well as for the mix of morning checks only (Day 1–5) and different daily checks (Day 4 plus 24 h check in the morning of Day 5).

The sample size estimation for this exploratory study was built on previous study experience from the investigating laboratory, assumed a fixed treatment effect including two levels and a random intercept on subject level (24 levels). A total of 24 subjects would detect a treatment difference at a two-sided 5% significance level with a statistical power of 80%—if the true difference between the treatment groups is 2.5 units (μV) in PtP-laser amplitude. This based on the assumption that the within-subject standard deviation of the response variable was 4.5 (μV).

The statistical analyses in this exploratory study were based on the evaluation of treatment-related differences to placebo.

These parameters were analysed using a linear mixed regression model. The regression included the classification variable treatment and the corresponding baseline value (pre-dose measurement on normal skin) as fixed effects and the intercept over the subjects as a random effect. All treatment differences with 95% confidence intervals were estimated from this model. The null hypotheses that these differences are equal to zero (no difference between active treatments and placebo) were tested at the 5% level against the two-sided alternatives. The efficacy analyses were performed using the full analysis set. All analyses were done by the statistical software package SAS™ Version 9.4 (SAS™, SAS Institute, Cary, NC, USA). The statistical model was fitted by the SAS procedure MIXED.

Since this was an exploratory study, no corrections for multiplicity were done.

For evaluation of the overall effect of ACD440 Gel versus placebo on the different parameters, the following analyses were conducted:

Corresponding to study objective (a), comparing the effect of ACD440 Gel versus placebo if applied on skin optimized for drug penetration (skin stripping, occlusive gel application) using vertex EEG LEPs and subjective pain rating, using an electronic 100 mm VAS, the respective AUCs were derived from all assessments at 1 h after application of study medication on Days 1–5, with the pre-application assessments as baseline reference.

Corresponding to study objective (b), comparing the effect of ACD440 Gel versus placebo in reducing the overall peak-to-peak (PtP) amplitudes of LEPs and VAS on normal skin, the respective AUCs were derived from all assessments at 1 h after application of study medication on Days 1–5, with the pre-application assessments as baseline reference.

In addition, study objectives (a) and (b) 24-h AUCs were evaluated comparing the effect of ACD440 Gel versus placebo over the 24 h from Day 4 to Day 5, the respective AUCs were derived from all assessments from 1 h after application of study medication on Day 4 up until and including the Day 5 assessment, thus encompassing 1, 6, 9 and 24 h after study medication on Day 4.

Corresponding to study objective (c), comparing the effect of ACD440 Gel versus placebo in reducing UVB irradiation induced hyperalgesia using LEPs, VAS and weighted needle threshold (WNT) to define (mechanical) pinprick hyperalgesia. 24-h AUCs were

evaluated comparing the effect of ACD440 Gel versus placebo over the 24 h from Day 4 to Day 5, the respective AUCs derived from all assessments from 1 h after application of study medication on Day 4 up until and including the Day 5 assessment, thus encompassing 1, 2, 3, 4, 5, 8, 11 and 24 h after study medication on Day 4.

In addition, after hierarchical testing of the efficacy variables, and based on the positive outcome of the overall AUC efficacy analyses, a set of post hoc pairwise comparison analyses on Day 4 assessments were conducted, in order to better understand any duration of action of the analgesic effects of ACD440 Gel versus placebo. For normal and optimized skin conditions (treatment areas 1–4), LEP PtP amplitudes and pain VAS assessments were explored, these time point analyses included the same assessment time points as included in the 24-h AUC. For the UVB-treated areas (treatment areas 5–6), LEP PtP amplitudes, pain VAS assessments and WNT were explored, time point analyses including the same assessment time points as included in the 24-h AUC.

Furthermore, to explore the potential difference in treatment effects on pain intensity (VAS 0–100) between normal and optimized skin conditions for the same parameters, these were explored for the 1-h time point on all treatment days.

3 | RESULTS

All 24 subjects (6 females, 18 males) aged from 24 to 61 years (mean age 45.8, SD 10.6 years, mean body weight 81.2, SD 14.0 kg, mean height 177.5, SD 8.6 cm, mean BMI 25.6, SD 3.0 kg/m^2) participated in all sessions, taking place between 12 January and 10 March 2021.

3.1 | Efficacy/pharmacodynamics (part 1)

3.1.1 | Effects on normal skin

Day 1–5 assessments: The AUC of changes over days 1–5 demonstrated substantial treatment effects as compared to placebo in favour of ACD440 for both the LEP PtP amplitude ($p < 0.001$) and the single LEP components (N2: $p < 0.001$; P2: $p = 0.013$). Also, subjective pain rating using VAS pain (0–100) was reduced significantly under treatment with ACD440 ($p < 0.001$). On day 4, the LEP PtP during 24 h showed analgesic effects of ACD440 versus placebo as described by changes in PtP amplitude already 1 h after treatment ($p = 0.002$). The effects were maintained over at least 9 h ($p < 0.001$). The effect on PtP was confirmed by significant treatment effects on the single LEP components N2 and P2 (AUC Day 4: $p < 0.001$). Pain intensity (VAS 0–100) was reduced in favour of ACD440 versus placebo, starting 1 h after application ($p = 0.005$) and the effects were maintained at the 6 and 9 h evaluation time points ($p < 0.001$ and $p = 0.005$, respectively) (Tables 1 and 2, Figure 4a).

3.1.2 | Effects on skin optimized for penetration

Day 1–5 assessments: The AUC of changes over days 1–5 confirmed the significant treatment effects as compared to placebo in favour of ACD440 for both the LEP amplitudes as well as subjective rating of pain using a VAS 0–100 (all $p < 0.001$). As expected, the magnitude of the positive drug effect (treatment difference vs. placebo) was more pronounced on skin optimized for penetration as compared to normal skin (i.e. reduction of VAS pain: 8.99 ± 2.51 on normal skin vs. 17.08 ± 2.51 on skin optimized for penetration). Day 4 assessments: The LEP PtP during 24 h at study day 4 confirmed the analgesic effects reported for normal skin. Treatment changes for all LEP amplitudes were substantially reduced in favour of ACD440 starting from the first observation time point at 1 h and are maintained over at least 9 h (overall: $p < 0.001$). Also pain intensity (VAS 0–100) on skin optimized for penetration was reduced in favour of ACD440 as compared to placebo, starting from the first observation point at 1 h after ACD440/placebo gel application ($p < 0.0001$). The effects were maintained at the 6 and 9 h evaluation time point ($p < 0.001$) (Tables 3 and 4, Figure 4b). The effect on pain intensity (VAS 0–100) in skin optimized for penetration compared to normal skin was significantly better at 1 h after application of ACD440 Gel on Day 1 ($p = 0.0064$), a difference that diminished over time, through Day 4 ($p = 0.0679$), and Day 5 ($p = 0.2342$) demonstrating the influence of penetration being important for a more rapid uptake (Table 5).

3.1.3 | Effects on UVB-irradiated skin

Effects of LEP PtP on UVB-irradiated skin were investigated during day 4, with the 24-h time point on day 5 (Table 6, Figure 5). UVB irradiation was performed 2 h after ACD440/placebo gel application. The analgesic effects of ACD440 versus placebo on UVB-irradiated skin showed effects immediately after UVB exposure ($p < 0.001$), lasting for up to 5 h after ACD440/placebo gel application (3 h after UVB irradiation; $p = 0.003$). The evaluation of the effects after irradiation needed to take into consideration the preceding

TABLE 1 Laser-evoked potential (LEP) amplitudes (peak-to-peak [PtP], N2, P2) and pain intensity (VAS 0–100) on normal skin: Least square means and comparison between ACD 440 and placebo of the AUC for Days 1–5.

Parameter	Medication	Estimate	Standard error	95% CI		*p*-Value
				Lower bound	Upper bound	
PtP (μV)	Placebo	20.21	1.08	18.03	22.39	
	ACD 440	16.09	1.07	13.95	18.24	
	Difference Placebo – ACD 440	4.12	1.09	1.93	6.30	<0.001
N2 (μV)	Placebo	10.16	0.55	9.05	11.27	
	ACD 440	7.67	0.55	6.56	8.77	
	Difference Placebo – ACD 440	2.49	0.55	1.39	3.59	<0.001
P2 (μV)	Placebo	10.17	0.62	8.92	11.41	
	ACD 440	8.42	0.60	7.20	9.63	
	Difference Placebo – ACD 440	1.75	0.68	0.38	3.12	0.013
VAS (0–100)	Placebo	45.83	2.79	40.22	51.45	
	ACD 440	36.84	2.80	31.21	42.47	
	Difference Placebo – ACD 440	8.99	2.51	3.93	14.05	<0.001

Abbreviations: AUC, area under the curve; CI, confidence interval; SE, standard error.

TABLE 2 Individual time point pairwise comparison Days 4–5 of laser-evoked potential (LEP) amplitudes (peak-to-peak [PtP]), pain VAS (0–100) on normal skin: Least square means and comparison between ACD 440 and placebo of the individual time points on Days 4–5.

Parameter	Time point (h)	Medication	Estimated difference	Standard error	95% CI		*p*-Value
					Lower bound	Upper bound	
PtP (μV)	1	Placebo vs. ACD440/normal	5.53	1.70	2.11	8.96	0.0021
	6	Placebo vs. ACD440/normal	7.66	1.55	4.55	10.78	<0.0001
	9	Placebo vs. ACD440/normal	7.63	1.35	4.91	10.35	<0.0001
Pain VAS (0–100)	1	Placebo vs. ACD440/normal	13.68	4.64	4.33	23.02	0.0050
	6	Placebo vs. ACD440/normal	16.42	4.40	7.56	25.27	0.0005
	9	Placebo vs. ACD440/normal	12.60	4.21	4.11	21.09	0.0045

sequential treatment effects that are being achieved on top of the effects achieved already on normal skin.

This is evidenced by an analgesic effect on WNT already present at the time of irradiation ($p = 0.049$). Results for the N2 and P2 LEP component confirm the results seen for PtP. The effects were more pronounced for the 'peripheral' N2 component (AUC: $p = 0.003$) as compared to the 'central' P2 component (AUC: $p = 0.032$). Pain intensity (VAS 0–100) on UVB-irradiated skin showed a significant reduction by ACD440 versus placebo (AUC 24 h: $p < 0.001$). Subjective pain intensity rating using the VAS (0–100) seemed here to be more sensitive to treatment changes on UVB-irradiated skin than to the LEP (AUC 24 h: PtP $p = 0.005$, N2 $p = 0.003$, P2 $p = 0.032$). Significant effects on pain intensity rating VAS (0–100) were seen already from the time of irradiation ($p = 0.023$), being maintained over the full observation period of 9 h after UVB and 11 h after ACD440/placebo gel application ($p = 0.020$). Significant treatment effects by ACD440 on mechanical hyperalgesia (increase in WNT) were reported starting as early as the time of UVB irradiation, that is, 2 h after ACD440/placebo gel application ($p = 0.020$) and were also confirmed at 1 h after UVB irradiation (3 h after ACD440 / placebo gel application), $p = 0.011$. The reduction of mechanical hyperalgesia over the 24 h (AUC) is also statistically significant larger for ACD440 versus placebo ($p = 0.049$).

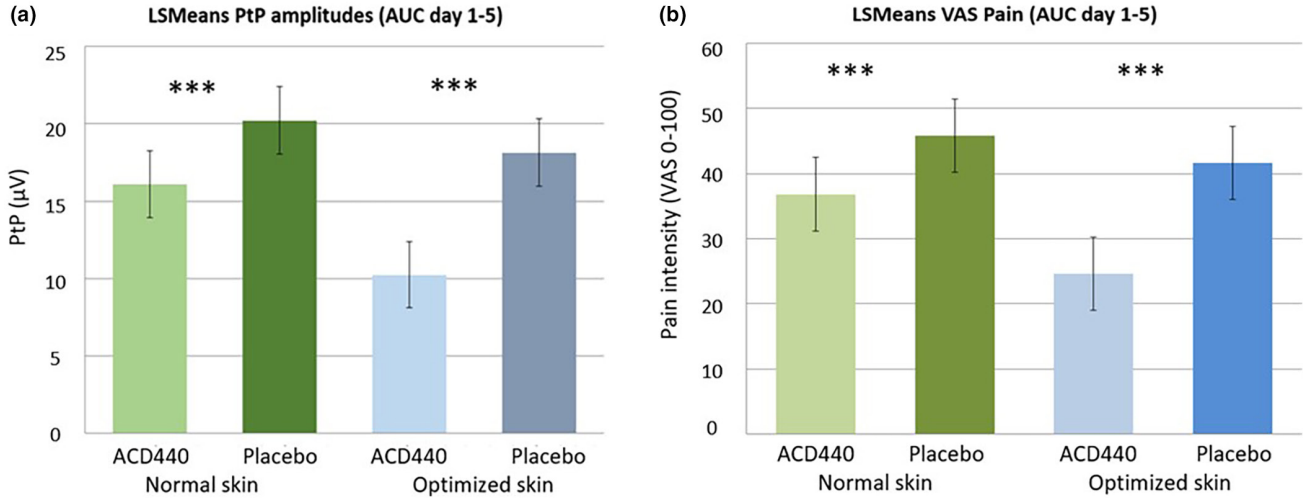


FIGURE 4 (a) ACD440 significantly reduced the objective peak-to-peak (PtP) amplitudes compared to placebo, assessed as area under the curve (AUC) for days 1–5, both under normal and optimized skin conditions. ***$p < 0.001$. (b) ACD440 significantly reduced the laser-evoked subjective VAS pain intensity (0–100), assessed as area under the curve (AUC) for days 1–5, compared to placebo, both under normal and optimized skin conditions. ***$p < 0.001$. Bars represent mean values; whiskers represent standard error. LSMeans depicts least square means.

TABLE 3 Laser-evoked potential (LEP) amplitudes (peak-to-peak [PtP], N2, P2) and pain intensity (VAS 0–100) on skin optimized for penetration: Least square means and comparison between ACD 440 and placebo of the AUCs for Days 1–5.

				95% CI		
Parameter	**Medication**	**Estimate**	**Standard error**	**Lower bound**	**Upper bound**	***p*-Value**
PtP (µV)	Placebo	18.13	1.08	15.94	20.31	
	ACD 440	10.25	1.07	8.10	12.39	
	Difference Placebo – ACD 440	7.88	1.09	5.68	10.08	<0.001
N2 (µV)	Placebo	8.80	0.55	7.68	9.91	
	ACD 440	5.03	0.55	3.93	6.14	
	Difference Placebo – ACD 440	3.76	0.55	2.65	4.87	<0.001
P2 (µV)	Placebo	9.22	0.62	7.98	10.46	
	ACD 440	5.22	0.60	4.00	6.43	
	Difference Placebo – ACD 440	4.01	0.68	2.64	5.37	<0.001
VAS (0–100)	Placebo	41.68	2.79	36.05	47.30	
	ACD 440	24.60	2.79	18.98	30.22	
	Difference Placebo – ACD 440	17.08	2.51	12.02	22.13	<0.001

Abbreviations: AUC, area under the curve; CI, confidence interval.

3.2 | Pharmacokinetics

In Part 1 (PD part) of the study, the once daily 1-h application (3 × 14 mg = 42 mg) on three 20 cm^2 skin surface areas for 5 consecutive days resulted in very low plasma concentrations. On Day 2 (24 h after 1st application and just before 2nd application), nine subjects out of 24 had quantifiable values, median 0.250 ng/mL (range 0.174–2.20 ng/mL) and 0.292 ng/mL (range 0.111–1.24 ng/mL) at 1 h post-dose on Day 5 (Figure 6a). On Day 9 (follow-up, i.e. 96 h after the 5th application), nine subjects out of 24 had detectable values, median 0.154 ng/mL (range 0.116–0.281 ng/mL, while 24 days after the last dose, none of the subjects had detectable plasma concentrations (LLOQ 0.05 ng/mL)).

In Part 2, (PK sub study), exposing 66.6 and 200 cm^2 skin surface area to a single dose (46.6 mg and 140 mg) of

TABLE 4 Individual time point pairwise comparison Days 4–5 of laser-evoked potential (LEP) amplitudes (peak-to-peak [PtP]), pain intensity (VAS 0–100) on optimized skin: Least square means and comparison between ACD 440 and placebo of the individual time points on Days 4–5.

Parameter	Time point (h)	Medication	Estimated difference	Standard error	95% CI Lower bound	95% CI Upper bound	*p*-Value
PtP (μV)	1	Placebo vs. ACD440/optimized	9.04	1.70	5.61	12.47	<0.0001
	6	Placebo vs. ACD440/optimized	8.77	1.55	5.65	11.89	<0.0001
	9	Placebo vs. ACD440/optimized	9.53	1.36	6.80	12.26	<0.0001
Pain VAS (0–100)	1	Placebo vs. ACD440/optimized	16.78	4.64	7.44	26.11	0.0007
	6	Placebo vs. ACD440/optimized	24.74	4.39	15.89	33.59	<0.0001
	9	Placebo vs. ACD440/optimized	15.49	4.21	7.01	23.97	0.0006

Abbreviation: CI, Confidence interval.

TABLE 5 VAS-Pain reduction at 1 h after application (normal and optimized): Pairwise comparisons of effect of ACD440 on normal versus optimized skin conditions, including estimate, standard error, *p*-value and 95% confidence interval.

Protocol time	Medication/condition	Estimate	Standard error	95% CI Lower bound	95% CI Upper bound	*p*-Value
Day 1	ACD 440/normal vs. optimized	12.46	4.36	3.68	21.25	0.0064
Day 2	ACD 440/normal vs. optimized	17.06	5.05	6.90	27.23	0.0015
Day 3	ACD 440/normal vs. optimized	15.26	6.42	2.32	28.20	0.0218
Day 4	ACD 440/normal vs. optimized	10.08	5.39	−0.77	20.93	0.0679
Day 5	ACD 440/normal vs. optimized	6.66	5.53	−4.47	17.79	0.2342

ACD440 Gel 14 mg/mL, all subjects had plasma concentrations above LLOQ (Figure 6b). The maximum average t_{max} following 46.6 mg and 140 mg was 8.9 h and 6.3 h, respectively, with an average C_{max} of 0.191 ng/mL (range 0.0653–0.309 ng/mL) and 0.492 ng/mL (range 0.111–0.992 ng/mL), respectively. The corresponding average AUC was 1.60 ng h/mL and 4.08 h ng/mL, respectively.

Overall, an approximately linear PK of ACD440 could be concluded from the dose corrected C_{max} and AUC-values, for the two dose levels studied. Elimination half-life, clearance, volume of distribution or AUC_{inf} could not be calculated due to many samples having non-detectable plasma concentrations.

3.2.1 | Safety

There were no adverse events reported, neither during the PD part of the study nor during the PK part. The SRS redness measure confirmed increased erythema intensity (inflammation) after UVB irradiation in line with the development of hyperalgesia but revealed no signs of additional redness induced by ACD440 after UVB exposure. There were no significant changes in vital signs, ECG variables or laboratory parameters.

3.2.2 | Skin reflection spectrometry (SRS)

The measurement of skin 'redness' (a-value of the Lab-system) in normal skin conditions did not display any ACD440 Gel effect versus the Placebo conditions. Both curves are fitting exactly over time, thus demonstrating a non-efficacy of the active ingredient versus placebo carrier on an erythema forming—to be considered as AE in normal skin.

4 | DISCUSSION

The results of the present proof-of-mechanism study in healthy subjects have demonstrated a significant effect of the topical gel formulation of the TRPV1 antagonist

TABLE 6 Sample time course of laser-evoked potentials (LEP) peak-to-peak (PtP) amplitude on skin irradiated with UVB: Least square means and comparison between ACD 440 and placebo of the individual time points and AUC over 24 h of Day 4.

PtP (μV)				95% CI		
Time after drug appl. (time after UVB irr.)	**Medication**	**Estimate**	**Standard error**	**Lower bound**	**Upper bound**	***p*-Value**
1 h	Placebo	15.15	1.23	12.60	17.70	
(−1 h)	ACD 440	12.82	1.23	10.28	15.37	
	Difference Placebo – ACD 440	2.33	1.57	−0.92	5.57	0.152
2 h	Placebo	19.06	1.91	15.10	23.01	
(0 h)	ACD 440	14.93	1.91	10.97	18.88	
	Difference Placebo – ACD 440	4.13	1.99	0.01	8.25	0.050
3 h	Placebo	21.50	1.80	17.78	25.22	
(+1 h)	ACD 440	11.81	1.80	8.09	15.53	
	Difference Placebo – ACD 440	9.69	2.17	5.20	14.18	<0.001
4 h	Placebo	22.39	1.56	19.16	25.63	
(+2 h)	ACD 440	15.28	1.56	12.04	18.51	
	Difference Placebo – ACD 440	7.12	1.97	3.05	11.18	0.001
5 h	Placebo	23.82	1.50	20.73	26.92	
(+3 h)	ACD 440	17.16	1.50	14.07	20.26	
	Difference Placebo – ACD 440	6.66	2.04	2.45	10.88	0.003
8 h	Placebo	25.49	2.08	21.19	29.80	
(+6 h)	ACD 440	22.12	2.08	17.81	26.43	
	Difference Placebo – ACD 440	3.37	1.71	−0.17	6.91	0.061
11 h	Placebo	24.30	2.20	19.75	28.86	
(+9 h)	ACD 440	23.34	2.20	18.78	27.89	
	Difference Placebo – ACD 440	0.97	1.98	−3.14	5.07	0.631
AUC 24 h	Placebo	24.41	1.66	20.98	27.85	
	ACD 440	20.30	1.66	16.86	23.73	
	Difference Placebo – ACD 440	4.12	1.33	1.35	6.88	0.005

Abbreviations: AUC, Area under the curve; CI, Confidence interval; SE, Standard error; UVB irr., UVB irradiation.

ACD440 in attenuating evoked pain under three different skin conditions: normal skin, skin optimized for drug penetration (by stripping and occlusive gel application) and UVB-inflamed skin. In this study, where peripheral nociceptor activation was induced by short laser pulses as pain processing readouts of the effects, that is, the amplitude of the LEP from vertex-EEG was reduced. In concordance, perceived pain intensity was significantly attenuated after application of ACD440 Gel. Thus, there was a marked reduction in sensitivity to heat stimuli (Madsen et al., 2014). After UVB irradiation, also pain elicited by mechanical stimuli was attenuated (Table 7).

Previous human experimental studies of oral TRPV1-antagonists have demonstrated a clear proof of mechanism, by reducing pain due to nociceptive provocation by heat or by local capsaicin administration (Chizh et al., 2007; Krarup et al., 2011; Schaffler et al., 2012; Sjögren et al., 2019). However, these orally administered compounds have also generated target-related adverse effects, such as increased core body temperature or even hyperthermia, as well as global insensitivity to heat, with a risk of acquiring minor

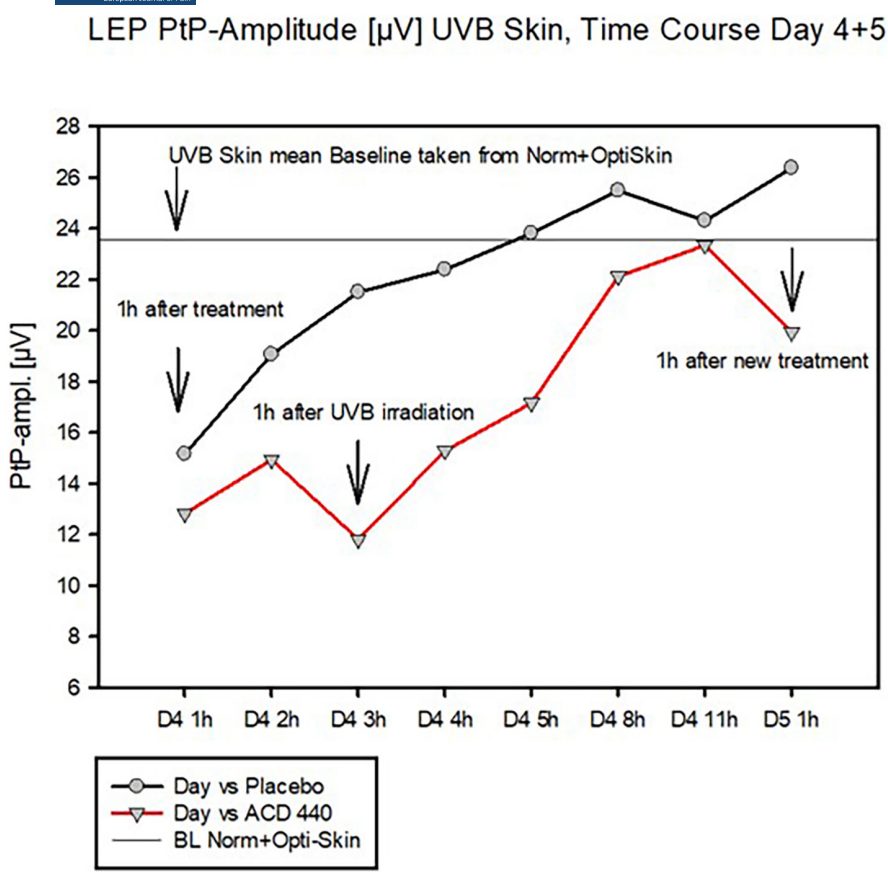


FIGURE 5 Time course of laser-evoked potential (LEP) peak-to-peak (PtP) amplitude on ultraviolet B (UVB)-irradiated skin conditions (of skin area 5 + 6) from Day 4 and morning of Day 5 for ACD440 Gel treatment and placebo. Times of drug application and UVB exposure are indicated, as well as the general baseline (BL) on normal skin conditions (straight line). Hyperalgesic development under UVB influence is to be seen—also taking into account already the preceding new early morning treatment on normal skin before irradiation. For descriptive and variance details of the respective measurement time points in the graph, see Table 3.

burns from ingesting hot beverages or from hot pans (Manitpisitkul et al., 2016). For this reason, further clinical development of oral products of this compound class has been halted. Instead, ACD440 was developed as a gel formulation, with the intent to provide an efficacious local treatment without the unwanted systemic side effects.

This approach gives the opportunity to evaluate the clinical usefulness of TRPV1 antagonism as a novel method for the treatment of localized pain conditions, for example, chronic peripheral neuropathic pain or acute peripheral pain of various origins, using a local administration paradigm.

It is noteworthy that already after only 1 h of topical application in these healthy subjects, a significant TRPV1 antagonistic effect was obtained, lasting for up to 9 h, thereby considerably outlasting the application time (1 h) of the compound on the skin. This indicates a rapid transdermal uptake and relevant depot effect in the skin. It also suggests that a twice daily application would be a feasible dosing schedule in a future clinical trial setting.

Several other compounds intended for pain relief have been investigated in the same pain models and in the same lab, applying identical procedures. Compared to studies in the same pain models (LEP and UVB-irradiation) with the oral administration of the TRPV1-receptor antagonist ABT-102 (0.5, 2 and 6 mg), as well with the opioid tramadol 100 mg and the selective COX-2 inhibitor etoricoxib 90 mg (Schaffler et al., 2012), ACD440 Gel is at least as efficacious in reducing LEP-induced PtP responses, LEP-induced subjective pain and mechanically induced pain after UVB irradiation.

Target-related side effects related to systemic exposure have been frequently reported in clinical trials of TRPV1-receptor antagonists, typically including at least some degree of hyperthermia, and a global loss of heat sensation, resulting in scalding and burns (Chizh et al., 2007; Manitpisitkul et al., 2016; Rowbotham et al., 2011). These can be avoided when administering the compound topically, resulting in very low systemic exposures to the drug. Topical treatment is thus favourable, as demonstrated by the very low plasma exposure detected in this clinical trial, expected to be several 100-fold lower than exposures giving rise to elevated heat pain thresholds in previous studies in healthy subjects (Manitpisitkul et al., 2016).

There are no published studies of TRPV1 antagonists in patients with neuropathic pain, neither central nor peripheral, despite animal studies supporting the hypothesis. This warrants considerations on the role of the TRPV1 receptor in a human chronic neuropathic pain state. Under normal conditions, the TRPV1 receptor is activated by heat, low pH and capsaicin. However, published nonclinical data demonstrate that under pathological, inflammatory or painful conditions, TRPV1 receptors may be subject to a 'phenotype switch' and that they also become sensitive to mechanical

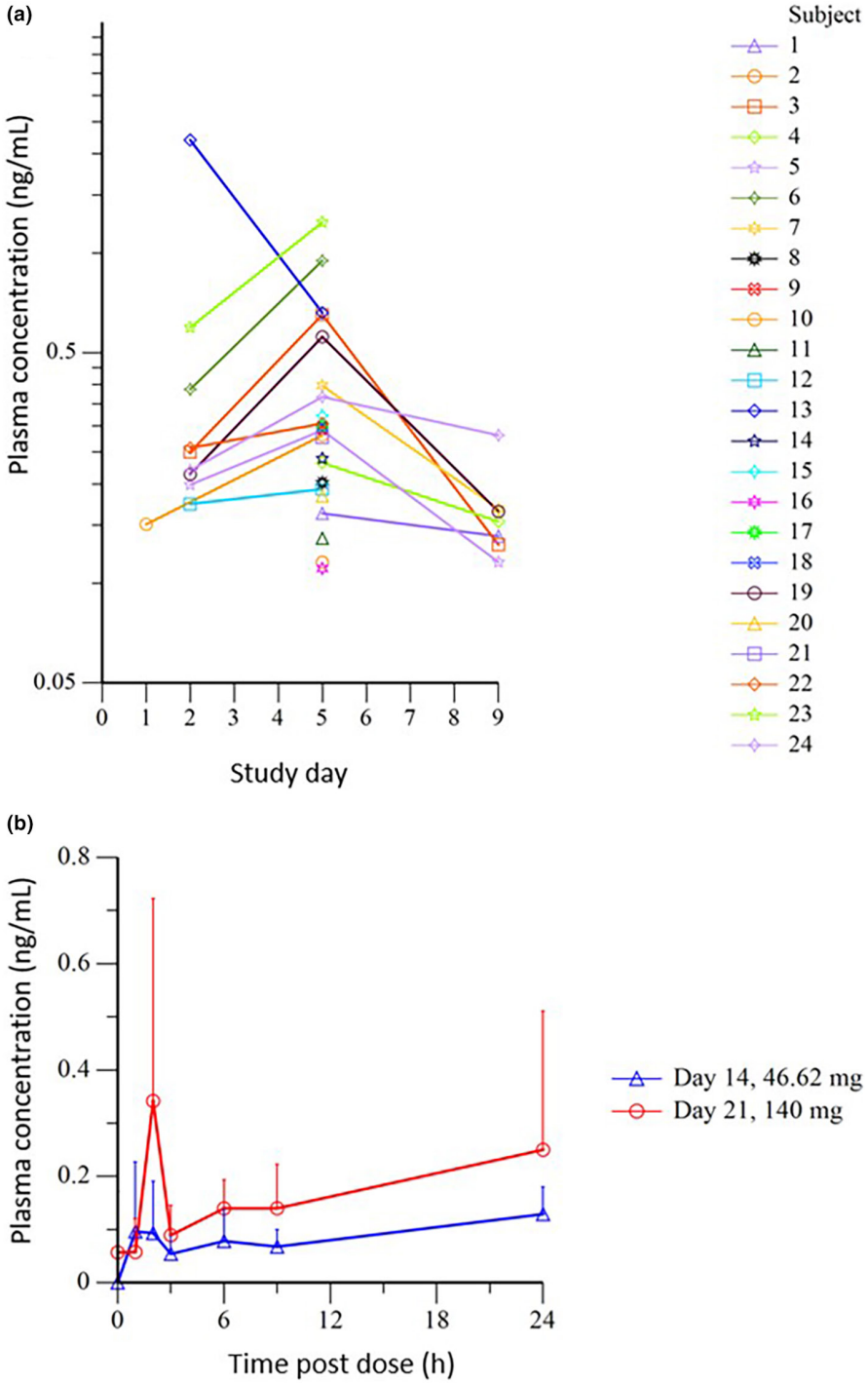


FIGURE 6 (a) Individual plasma concentration of ACD440 in ng/mL over time for all 24 subjects of study part 1, Days 1, 2, 5 and 9 after topical administration of ACD440 (logarithmic *y*-axis). (b) PK substudy: Mean (SD) concentration of ACD440 in plasma over time for eight subjects after topical administration of ACD440. Lowest level of quantification (LLOQ) was 0.05 ng/mL.

stimuli (Chang et al., 2021; Werland et al., 2021). Thus, it is hypothesized that a TRPV1 receptor antagonist, such as ACD440, could be effective across the thermal and the mechanical sensory hyperalgesia spectrum. This is congruent with internal findings of the present study, where also an increased mechanical threshold was demonstrated after topical application of ACD440.

ACD440 is currently under clinical development for the treatment of peripheral neuropathic pain with sensory hypersensitivity, based on the presumption that epidermal presence of thin fibre nociceptors is a prerequisite for target engagement. Recently, several companies have again developed an interest in the TRPV1 receptor as a target for the treatment of pain, applying topical administration. Qutenza®, an 8% capsaicin (a TRPV1-receptor agonist) patch, has been on the market for over a decade. Being a high concentration agonist at the TRPV1 receptor (also known as the capsaicin receptor) exerts its action by activating the receptor, which makes application in itself painful, and treatment should be conducted with safety precautions within a healthcare facility. The duration of high concentration local capsaicin treatment is longer than the application time, due

TABLE 7 Overview of the main efficacy results across all three skin conditions as pairwise comparisons of placebo versus ACD440 and their resulting significance levels.

Differences placebo/ACD	Laser-evoked potential peak-to-peak ampl. (μV)		Visual analogue scale (mm)		Weighted needle threshold[a] (mN)		Skin reflection spectroscopy (−)	
Parameter	**Estimate (SE)**	***p*-Value**	**Estimate (SE)**	***p*-Value**	**Estimate (SE)**	***p*-Value**	**Estimate (SE)**	***p*-Value**
Normal skin								
AUC Days 1–5	4.12 (1.09)	<0.001	8.99 (2.51)	<0.001	n.d.	n.d.	n.d.	n.d.
AUC Day 4	6.76 (1.18)	<0.001	13.20 (3.33)	<0.001	n.d.	n.d.	n.d.	n.d.
Skin optimized for penetration								
AUC Days 1–5	7.88 (1.09)	<0.001	17.08 (2.51)	<0.001	n.d.	n.d.	n.d.	n.d.
AUC Day 4	9.06 (1.19)	<0.001	18.66 (3.33)	<0.001	n.d.	n.d.	n.d.	n.d.
UVB-irradiated skin								
AUC Day 4	4.12 (1.33)	0.005	8.48 (2.23)	<0.001	−8.11 (3.86)	0.049	0.06 (0.23)	0.801

Abbreviations: AUC, area under the curve; n.d., not done; SE, standard error.

[a]In WNT, an increase of the threshold is the positive drug effect; therefore, the difference Plc/ACD 440 is negative.

to the mechanism of action, reducing the epidermal innervation and nociceptor function (Bley, 2013). The recovery of the epidermal innervation is what makes up the duration of action, of up to 12 to even 48 weeks (Mou et al., 2014). In contrast, a TRPV1-receptor antagonist does not cause pain on application and its duration of action per application is only dependent on its local presence in the skin tissue. Furthermore, the treatment can easily be handled by the patients at home. The duration of effect, as reported in the findings of this study, would likely require a twice daily application. The treatment concept of antagonizing the activation of the receptor has after a quiescence of a decade quite recently been raised again, and for several different indications, for example, such as Novartis' libvatrep, reporting positive data in acute and chronic eye pain (Stasi et al., 2022; Thompson et al., 2023). The strengths of the current study included the previous validation of the models, the multitesting in the healthy subjects. Furthermore, simultaneous testing across different exposure conditions avoided any timely period effects by inducing any carryover effects or placebo or nocebo effects, which all may often occur when there is some time between the experimental sessions. The study included testing of ACD440 Gel in accordance with regulatory guidance for products intended for topical use. Furthermore, the masking of the subjects and of the investigator conducting the study assessments enabled adequate blinding of the study treatments. The plasma exposure of ACD440 following topical administration of up to 140 mg ACD440 Gel did not exceed 1 ng/mL, far below the exposure expected to induce any systemic adverse events.

Limitations of any study in healthy subjects are that it can never fully predict the clinical effect in the intended patient population. Even though target engagement was clearly demonstrated (analgesia/anti-hyperalgesia), this is an effect in a normal sensory nervous system, without the pathophysiological changes occurring in the patient suffering from peripheral neuropathic pain (Facer et al., 2007). Therefore, drawing any conclusions around clinically relevant effects in patients suffering chronic peripheral neuropathic pain should be done with caution. Furthermore, though the models used are well validated with many compounds of different classes, these models are limited to exploring effects on thermo-nociceptive, mechanical stimuli and effects on an UVB-mediated inflammatory response (Schaffler et al., 2012, 2017).

5 | CONCLUSIONS

ACD440 Gel (14 mg/g) significantly reduced the PtP amplitude of LEPs induced by radiant heat laser stimulation of normal skin and skin optimized for penetration versus

the placebo gel application, as well as the corresponding perceived subjective pain intensity. Furthermore, both laser-induced pain and mechanically induced pain by weighted needle pressure were also attenuated by ACD440 Gel compared to placebo in UVB-irradiated inflamed skin. Effects were extended long after removal of the gel. Application of ACD440 Gel in an efficacious concentration on a clinically relevant body surface area demonstrated extremely low plasma concentrations, with many plasma samples being below the lowest level of quantification. There were no safety findings, neither locally to the skin nor as reported systemic adverse events. ACD440 Gel has demonstrated proof of mechanism by target engagement supporting further clinical development in relevant patient populations. The present results warrant further development of ACD440 Gel as a topical treatment for local clinical pain conditions, including chronic peripheral neuropathic pain, or other acute or chronic skin pain conditions.

AUTHOR CONTRIBUTIONS

MR, TP and KS (as PI) contributed to study design, study execution, data and result interpretation and critical review of the manuscript. MMH contributed to study design, pharmacokinetic evaluation, data and result interpretation and critical review of the manuscript. MS contributed to data and result interpretation and is the main author of the manuscript. All authors have commented on the manuscript.

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CONFLICT OF INTEREST STATEMENT

MS is an employee of AlzeCure Pharma AB; MR and MH are consultants working for AlzeCure Pharma AB; KS is the principal investigator and owner and TP the co-investigator of the clinical CRO HPR Dr. Schaffler GmbH.

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