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Investigation of the predictive validity of laser-EPs in normal, UVB-inflamed and capsaicin-irritated skin with four analgesic compounds in healthy volunteers

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Keywords laser-evoked potentials (LEPs), nociceptive/hyperalgesic/analgesic efficacy, phase I, UVB- and capsaicin-irritated skin

AIMS

The aim of the present study was to assess the predictivity of laser-(radiant-heat)-evoked potentials (LEPs) from the vertex electroencephalogram, using an algesimetric procedure, testing the anti-nociceptive/anti-hyperalgesic effects of single oral doses of four marketed analgesics (of different compound classes) *vs.* placebo, in healthy volunteers with three skin types.

METHODS

This was a randomized, placebo-controlled, single-blind, five-way-crossover trial. Twenty-five healthy male/female Caucasians were included (receiving celecoxib 200 mg, pregabalin 150 mg, duloxetine 60 mg, lacosamide 100 mg or placebo) in a Williams design, with CO_2 laser-induced painful stimuli to normal, ultraviolet (UV) B-inflamed and capsaicin-irritated skin. LEPs and visual analogue scale ratings were taken at baseline and hourly for 6 h postdose from all three skin types.

RESULTS

In normal skin, the averaged postdose LEP peak-to-peak-(PtP)-amplitudes were reduced by pregabalin (−2.68 μV; 95% confidence interval (CI) −4.16, 1.19) and duloxetine (−1.73 μV; 95% CI −3.21, −0.26) but not by lacosamide and celecoxib *vs.* placebo. On UVB-irradiated skin, reflecting inflammatory pain, celecoxib induced a pronounced reduction in LEP PtP amplitudes *vs.* placebo (−6.2 μV; 95% CI −7.88, −4.51), with a smaller reduction by duloxetine (−4.54 μV; 95% CI −6.21, −2.87) and pregabalin (−3.72 μV; 95% CI −5.40, −2.04), whereas lacosamide was inactive. LEP PtP amplitudes on capsaicin-irritated skin, reflecting peripheral/spinal sensitization, as in neuropathic pain, were reduced by pregabalin (−3.78 μV; 95% CI −5.31, −2.25) and duloxetine (−2.32 μV; 95% CI −3.82, −0.82) but not by celecoxib or lacosamide *vs.* placebo, which was in agreement with known clinical profiles. Overall, PtP amplitude reductions were in agreement with subjective ratings.

CONCLUSIONS

LEP algesimetry is sensitive to analgesics with different modes of action and may enable the effects of novel analgesics to be assessed during early clinical development.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Diverse compound classes (other than nonsteroidal anti-inflammatory drugs and narcotics) have demonstrated efficacy in animal, human phase I and clinical pain studies.
- Laser- (radiant heat) evoked potentials (LEPs) and pain visual analogue scales, approved in previous studies, have been used specifically to evaluate the anti-nociceptive/hyperalgesic effects of four marketed compounds (of different classes) *vs.* placebo in normal, ultraviolet B-inflamed and capsaicin-irritated skin.

WHAT THIS STUDY ADDS

- The present results using the LEP model indicated that it is feasible to differentiate between the efficacies of diverse compound classes – with regard to thermal hyperalgesia – in a single-dose paradigm.
- The algesimetric model showed reproducibility and validity, and correlated with clinical outcomes.
- The suitability and predictivity of the model was confirmed in small numbers of normal healthy subjects.

Tables of Links

TARGETS	
Enzymes [2]	**Voltage-gated ion channels** [4]
COX-2	Votage-gated sodium channels
Transporters [3]	Voltage-gated calcium channels
NET	
SERT	

LIGANDS
Celecoxib
Duloxetine
Lacosamide
Pregabalin

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2–4].

Introduction

Chronic pain, a frequent pathological state associated with a decreased quality of life, reduces functionality, causes temporary or permanent unfitness for work and represents a significant public health burden. Unfortunately, although there is a high unmet medical need for novel effective pain treatments, the development of new analgesic compounds remains a major challenge for the pharmaceutical industry. Indeed, the extrapolation of the analgesic effects obtained with novel pharmacotherapies in evoked pain animal models to complex human pain states has so far been associated with a high rate of failure in relatively large and costly patient trials [5, 6].

In order to assess the analgesic potential of a new drug candidate in early clinical development (ECD), to inform subsequent clinical study designs and to support decision making before entering into large and costly phase II and III studies, several translational experimental human pain models have been proposed. In general, these models build on the standardized activation of nociceptive pathways in healthy volunteers or patients, combined with quantitative and objective recordings of the evoked pain responses [7]. For example, it has been shown that specific CO_2 laser stimulations can be repeatedly applied to normal [8, 9] and hyperalgesic (ultraviolet (UV)-irradiated/inflamed- and capsaicin-irritated skin) [8–12] skin types in healthy volunteers to generate laser-evoked potentials (LEPs), recorded from the vertex electroencephalogram (EEG) by filtering and averaging triggered responses.

LEPs from normal skin may enable the assessment of stable intradiurnal perception/processing conditions and/or sole antinociceptive properties of compounds [8, 9]. LEPs from UVB-irradiated skin primarily mimic acute, post-traumatic and/or postoperative/inflammatory pain (mainly peripheral hyperalgesia) [8, 9]. Finally, LEPs from capsaicin-irritated skin are thought to be useful for investigating conditions resembling neuropathic pain, owing to ongoing nociceptive input at the spinal level (inducing mixed peripheral–spinal/central hyperalgesia) [8, 10, 13, 14].

It has been shown that various classes of drugs with antinociceptive and antihyperalgesic properties – depending on their mechanisms of action – reduce the amplitude of LEP EEG peak-to-peak (PtP) components, when compared with placebo, in healthy volunteers [8–11, 15, 16]. In addition, the results from a meta-analysis have shown that the PtP amplitude reductions obtained with marketed analgesics correlated well with scores obtained using subjective self-reporting pain rating scales such as the visual analogue scale (VAS) [8]. This further suggests the potential usefulness of this objective procedure in the assessment of candidate analgesics with new modes of action (MoA) during ECD [17]. Furthermore, the use of validated contact-free thermonociceptive laser stimulation technology in algesimetry is supported by the European Federation of Neurological Societies (EFNS) guidelines, describing the use of laser technology as a reliable tool for assessing nociceptive pathways in humans [16, 18].

The present study was designed to assess further the usefulness of the LEP paradigm in ECD. To that end, the effects of four marketed analgesics from different compound classes (celecoxib, pregabalin, duloxetine and lacosamide), with well-established clinical profiles, were tested *vs.* placebo in a single-dose paradigm on different skin types (normal, UV-irradiated/inflamed, and capsaicin-irritated skin) in the same cohort of healthy human volunteers.

Methods

Study participants

The protocol, subject information and consent form were approved by the relevant ethics committee (Ethics Committee of the Bavarian Chamber of Physicians (BLÄK), Munich, Germany – EC-No. 12048) and by the German competent authority (German Federal Health Authority (BfArM), Bonn, Germany – BfArM-No. 4038215). The study was conducted at a single site (Human Pharmacodynamic Research GmbH, Munich, Germany) in accordance with the principles of the Declaration of Helsinki and its current amendments, the federal clinical trial directives (based on German regulations, German Drug Law (AMG), the International Conference on Harmonization guidelines CPMP/ICH/135/95) and the guidelines for good clinical practice. The trial was registered under the EudraCT number 2012–003202-26.

Twenty-five healthy subjects (nine female and 16 male), aged between 20 and 52 years [mean age 35.8 years, standard deviation (SD) 9.1, median 34.0; mean body mass index (BMI) 24.5, SD 2.7, median 24.7) participated in the study. Following written informed consent, the subjects were enrolled based on inclusion and exclusion criteria consisting of prestudy physical examination, medical history, electrocardiogram (ECG) recording, vital signs and clinical laboratory tests. Suspected allergy to the reference drugs or placebo components, hypersensitivity to UVB (e.g. photoallergy), acne, widespread tattoos, scars or any pathogenic dermatological condition at the site of exposure to the laser, capsaicin and UVB were among the exclusion criteria.

Subjects who did not consent to abstain from using topical drugs or cosmetics at the site of exposure to the laser, capsaicin and UVB, and from sunbathing from 2 weeks prior to the first study drug administration until the end of treatment were not allowed to participate in the study. Only subjects with skin types II–IV, according to Fitzpatrick, were used (covering about 90% of the skin types of the European population).

For female subjects who were not postmenopausal or sexually abstinent, or whose partners were not vasectomized, inclusion criteria included the use of physical contraceptive barriers (e.g., condoms) in addition to adequate hormonal contraceptives. Female subjects who were pregnant or breastfeeding were excluded.

Study design and treatments

This was a randomized, single-blind, single-centre, exploratory clinical study in which each subject received single doses of analgesics of different classes [i.e. celecoxib (200 mg Celebrex®), pregabalin (150 mg Lyrica®), duloxetine (60 mg Cymbalta®) and lacosamide (100 mg Vimpat®)] or placebo in a five-way (intraindividual) crossover design. Analgesics were prepacked in opaque individual bottles and labelled with the subject randomization number and treatment period, in accordance with the randomization scheme. An external independent pharmacy was responsible for the packaging and labelling of trial medications. Doses of analgesics were selected based on recommended standard and efficacious clinical doses.

Schedule of study assessments

Screening took place between 21 and 2 days before first study drug administration. Following screening, eligible subjects were randomized to one of the 10 possible treatment sequences, determined using a Williams design [19]. It was planned that each subject attended five treatment periods, separated by a washout period of 7 days between each drug administration. The end-of-trial visit took place immediately after completion of the final treatment period. The total duration of the study was approximately 6–7 weeks, including screening.

On the assessment day for each treatment period, the study drug was administered directly from the prepared vials into the oral cavity (to avoid presenting any visual cues for the subject and investigator, to ensure blinding) together with 150 ml of tap water after an overnight fasting period of 10 h (controlled by a predose capillary blood glucose check in the morning) and then 3 h after a small standard breakfast. On this day, a small standard snack and a standard meal were served immediately after completion of the 2 h and 4 h postdose LEP and VAS postlaser pain assessments.

Preparatory procedures at screening

At screening, six different skin squares (1 cm × 1 cm each) from the back of each subject were exposed to six ascending doses of UVB [using invisible range 290–320 nm; UVB narrow-band Dermalight® 80, with an emission peak at 311 nm (Dr Hönle Medizintechnik, GmbH, Kaufering, Germany)] in order to determine the minimal erythema dose (MED) – that is, the minimal UVB dose which produces a clearly discernible erythema. After a development time of 6–8 h, the visual identification of the square area showing the first clearly discernible rectangular erythema was used to determine the individual MED (i.e. resulting in individual exposure times of approximately 2–6 min for the later twofold MED application on the main assessment days, depending on the individual skin characteristics).

In addition, an individual (thermonociceptive) CO_2 laser pain threshold (LPT) – induced by Synrad Infrared Gas Laser Model E48–1/–10 W (Synrad Inc., North Bothell, WA, USA) (laser emission in the far infra-red spectrum at 10 200 to 10 600 nm, beam diameter 3.5 mm) – was determined by the application of a slowly increasing laser beam intensity to the normal skin of each participant until they felt a 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 the UV dose were both kept constant over the entire study period for each individual.

Skin sensitization and laser-induced thermonociception procedures during treatment periods

On the morning of the main assessment day for each treatment period, a twofold individual MED was applied (invisible range 310–315 nm; UVB narrow-band Dermalight® 80, with an emission peak at 311 nm) to a defined area of the skin on the back (5 × 5 cm each), 2 h before study drug administration. After UVB exposure, capsaicin (500 µl as a standardized 1% alcoholic extract; Extrakt Chemie, Stadthagen, Germany) was applied as a topical occlusive treatment for 30 min to a contralateral circular skin area (5.5 cm in diameter) in each subject 1 h 50 min before each drug administration. For each treatment period, skin areas (untreated; also referred to as normal, UVB inflamed and capsaicin irritated) were randomly switched, using different dermatomes and contralateral sites, to avoid a possible change in skin sensitivity by re-exposure, as a result of adaptation or overstimulation.

Thermonociception was induced by the application of CO_2 laser stimuli to normal (untreated) and sensitized skin (UVB inflamed or capsaicin irritated), at predefined time points, before (for baseline assessments) and after treatments. At each time point, the normal skin evaluation was always performed first. Laser stimuli to normal skin were also set at −2 h 30 min prior to drug administration as a warm-up (not evaluated). Stimuli to UVB-inflamed or capsaicin-irritated skin were induced at −30 min and −5 min, serving as 'wind-up' sessions for hyperalgesia development ('kindling'); the outcome was not evaluated. Baseline measurements for the LEPs and VAS postlaser pain were determined before dosing at −2 h 5 min and −1 h 20 min as baseline for UVB- (on untreated skin) and capsaicin-treated skin, respectively. Further LEPs and VAS postlaser pain assessments from UVB- and capsaicin-treated skin were performed following study drug administration (0:00 h) at predefined time points (1 h, 2 h, 3 h, 4 h, 5 h and 6 h).

Effects of laser-induced thermonociception on vertex EEG and pain perception recordings

At selected time points, thermonociception – induced by far infrared CO_2 laser stimuli (with fixed individual intensities determined at screening; mean laser intensity applied in n = 25 subjects at about 110 mJ per stimulus, with random interstimuli intervals of 4–8 s, and stepwise changes to another location of stimulation by about 3 mm) to normal, hyperalgesic UVB- and capsaicin-irritated skin – was objectively and quantitatively assessed by measuring the PtP amplitudes of the N2 and P2 evoked potential (EP) components, assessed from vertex EEG recordings (Figure 1).

EP signals were automatically obtained via programmable bio-amplifiers by online real-time averaging of 12 artefact-free, Gaussian phase-free filtered vertex EEG sections (EEG leads vertex/C_z *vs.* right mastoid/C_{br} – after automatic rejection of blinks, facial electromyogram influences of EMG activity and EEG baseline drifts; filter setting 0.15–30 Hz), sampled with a digitization rate of 512 Hz, following laser stimuli of 60 ms duration each. The antinociceptive/antihyperalgesic effects of study medications exist in case of reductions in the resulting EP signal amplitudes *vs.* placebo [16].

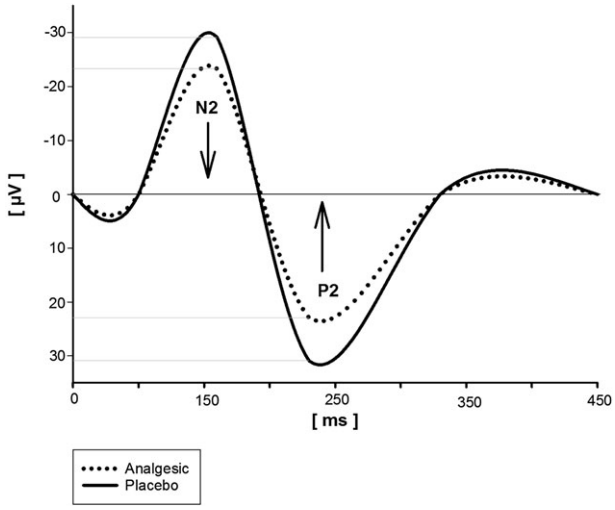

Figure 1

Principal N2 and P2 components of laser-evoked potentials with a typical overlay of analgesic *vs.* placebo waveforms

Thermonociceptive perception was also assessed subjectively immediately after each LEP session, using an electronic 100 mm VAS on a personal tablet computer.

Throughout the sessions, in order to avoid any external noise disturbances, to increase and stabilize subjects' vigilance and to distract them from pain stimulation and pain sensation expectancy, subjects were exposed to 'white noise' via earphones (with a sound pressure of 85 dBA) and had to carry out a continuous pursuit tracking task on a computer screen.

Safety and compliance assessments

Routine safety and compliance assessments – including clinical laboratory evaluations, vital signs, ECG, physical examination, urine drug screen, alcohol and CO/smoking screen and urine pregnancy test – were performed at screening.

The urine drug screen, alcohol and CO/smoking screen, urine pregnancy test and medical check (vital signs, including temperature, blood pressure and heart rate) were also conducted at the beginning of each treatment period.

Adverse events (AEs) were monitored throughout the study. All AEs were assessed with respect to their seriousness, severity, timing, duration, relation to treatment, the action taken and the outcome.

Measurement of study drug concentrations in the plasma

To assess the exposure levels of the respective treatments, two control blood samples were collected at 2 h and 4 h postdose, after completion of the scheduled LEP, VAS and safety assessments.

Following blood collection, plasma was separated and stored at −20°C pending analysis. Study drug (control) concentrations were determined using a validated liquid chromatography tandem-mass spectrometry (LC–MS/MS) method.

Statistical analysis

The statistical analysis was based on a linear mixed-effects model for the analysis of repeated-measure crossover designs [20]. The model was fitted to the PtP amplitude and VAS pain score obtained after laser stimulation of normal, UVB- and capsaicin-irritated skin. The model included the classification variables treatment (celecoxib 200 mg, pregabalin 150 mg, duloxetine 60 mg, lacosamide 100 mg or placebo), medication period (1 to 5), treatment sequence (1 to 10, according to the Williams design) and baseline value (period-specific predose measurement) as fixed effects. By adding the treatment sequence as a fixed effect, a cross-level bias was avoided [21]. In a first step, exploratory statistical analyses for averaged LEP variables and VAS postlaser stimulation pain scores were performed to obtain a summary treatment effect over all session time points (see Table 1). In a further analysis, the raw values for all session time points were investigated. For all subjects, the intercept and the effects at each of the session time points (1 to 6 h p.a.) were modelled as random effects. Moreover, to allow for different time courses of effects, the interaction term for treatment by session was included in the model. The covariance structure for the random effects was unrestricted. The homogeneity assumption for the error variance within each treatment was made. This enables a more detailed exploratory consideration with regard to the different session time points (see Table 2). Owing to the exploratory nature of the study, no adjustment for multiple testing was made.

All analyses were performed using the statistical software package SAS Version 9.2 (SAS Institute, Cary, NC, USA). Data are presented as adjusted means (least square means) of averaged PtP amplitudes and averaged VAS pain scores over the 6 h postdose interval for the treatment groups, as well as adjusted treatment differences and their corresponding 95% confidence intervals (CIs).

Safety assessments were only listed and analysed descriptively.

Sample size determination

It was anticipated that a five-way crossover study performed in 25 subjects would be able to detect a treatment difference at a two-tailed significance level of 5%, with a statistical power of 80%, if the true difference in LEP were to be 2.1 μV amplitude units between the treatment groups. This was based on the assumption that the within-subject standard deviation of the response variable would be 6.5, which was estimated for the placebo group based on the pooled data of various previous studies performed at the study site.

Results

Among the 27 healthy volunteers screened at the study site, 25 were eligible (according to inclusion and exclusion criteria) and were randomized to receive single doses of analgesics (celecoxib, pregabalin, duloxetine and lacosamide) and placebo in a crossover fashion. One subject decided to withdraw his consent for personal reasons after he had received two of the five study treatments (i.e. pregabalin and

Table 1

Least square means [+ 95% confidence interval (CI)] and mean differences from placebo for averaged N2–P2 peak-to-peak (PtP) amplitudes of laser-evoked potentials (LEP) (in μV) and visual analogue scale (VAS) scores (in mm) as measured over the 6 h post administration (p.a.) period in normal, ultraviolet (UV) B-irradiated and capsaicin-irritated skin in healthy subjects treated with single oral doses of celecoxib 200 mg, pregabalin 150 mg, duloxetine 60 mg, lacosamide 100 mg or placebo

		LEP PtP amplitude [μV]		VAS postlaser pain score [mm]	
Skin condition	**Treatment**	**Least square means (95% CI)**	**Mean difference from placebo (95% CI)**	**Least square means (95% CI)**	**Mean difference from placebo (95% CI)**
Normal skin	Placebo	24.3 (20.7, 28.0)	–	44.8 (31.0, 58.5)	–
	Celecoxib	24.3 (20.7, 27.9)	–0.1 (−1.5, 1.4)	**41.8** (28.1, 55.5)	**–2.9*** (−5.2, −0.7)
	Pregabalin	**21.7** (18.0, 25.3)	**–2.7**** (−4.2, 1.2)	**35.7** (21.9, 49.4)	**–9.1**** (−11.3, −6.9)
	Duloxetine	**22.6** (19.0, 26.2)	**–1.7*** (−3.2, −0.3)	42.5 (28.8, 56.3)	–2.2 (−4.5, 0.0)
	Lacosamide	23.8 (20.2, 27.5)	–0.5 (−2.0, 1.0)	42.7 (28.9, 56.4)	–2.1 (−4.4, 0.2)
UVB-irritated skin	Placebo	30.2 (27.7, 32.7)	–	57.6 (49.1, 66.2)	–
	Celecoxib	**24.0** (21.6, 26.5)	**–6.2**** (−7.9, −4.5)	**49.5** (41.0, 58.1)	**–8.1**** (−10.4, −5.8)
	Pregabalin	**26.5** (24.0, 29.0)	**–3.7**** (−5.4, −2.0)	**48.9** (40.4, 57.5)	**–8.7**** (−11.0, −6.4)
	Duloxetine	**25.7** (23.2, 28.1)	**–4.5**** (−6.2, −2.9)	**53.4** (44.9, 62.0)	**–4.2**** (−6.5, −1.9)
	Lacosamide	29.1 (26.7, 31.6)	–1.1 (−2.8, 0.6)	57.0 (48.3, 65.5)	–0.7 (−3.0, 1.6)
Capsaicin-irritated skin	Placebo	23.4 (19.4, 27.4)	–	55.4 (40.9, 69.8)	–
	Celecoxib	22.1 (18.1, 26.1)	–1.3 (−2.8, 0.2)	53.2 (38.8, 67.7)	–2.1 (−4.6, 0.3)
	Pregabalin	**19.6** (15.6, 23.6)	**–3.8**** (−5.3, −2.3)	**47.8** (33.3, 62.2)	**–7.6**** (−10.0, −5.2)
	Duloxetine	**21.1** (17.1, 25.1)	**–2.3*** (−3.8, −0.8)	**52.4** (37.9, 66.8)	**–3.0*** (−5.4, −0.6)
	Lacosamide	22.3 (18.4, 26.3)	–1.1 (−2.6, 0.5)	**51.6** (37.2, 66.1)	**–3.7*** (−6.3, −1.2)

Statistically significant values (for least square means and mean differences from placebo) are in bold type

*$P \leq 0.05$ *vs.* placebo; **$P \leq 0.001$ *vs.* placebo

Table 2

Least square means [+ standard error of the mean (SEM)] over the period 0 to 6 h post administration (p.a.) (hourly time course including baselines) for averaged N2–P2 peak-to-peak (PtP) amplitudes of laser-evoked potentials [μV] and visual analogue scale (VAS) pain scores (in mm) as measured on normal, ultraviolet (UV) B-irradiated and capsaicin-irritated skin in healthy subjects treated with single oral doses of celecoxib 200 mg, pregabalin 150 mg, duloxetine 60 mg, lacosamide 100 mg or placebo. Normal skin baseline values were used for predose measurements of both UVB-irradiated and normal skin conditions

	Predose		1 h p.a.		2 h p.a.		3 h p.a.		4 h p.a.		5 h p.a.		6 h p.a.	
Treatments	**mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**
PtP amplitude in normal skin (μV)														
Placebo	28.3	2.1	25.7	2.6	24.2	2.6	24.1	2.6	23.5	2.6	25.4	2.7	23.1	2.6
Celecoxib 200 mg	25.5	2.3	26.4	2.6	24.3	2.6	23.3	2.6	24.5	2.6	23.7	2.7	23.6	2.6
Pregabalin 150 mg	24.9	2.2	25.0	2.6	21.2	2.5	21.0	2.6	21.0	2.6	21.3	2.7	20.4	2.6
Duloxetine 60 mg	25.3	2.4	25.3	2.6	23.1	2.5	22.3	2.6	22.5	2.6	21.6	2.7	20.8	2.6
Lacosamide 100 mg	25.8	2.5	25.8	2.6	24.0	2.6	24.9	2.6	24.6	2.6	22.2	2.7	21.7	2.6
PtP amplitude in UVB-irradiated skin (μV)														
Placebo			29.0	2.0	30.2	1.9	30.9	1.9	29.4	1.8	30.3	2.1	31.6	1.9
Celecoxib 200 mg			24.8	1.9	23.1	1.9	23.6	1.9	24.2	1.8	23.8	2.1	24.6	1.9
Pregabalin 150 mg			25.7	1.9	27.2	1.9	27.4	1.9	26.3	1.8	26.0	2.0	26.5	1.8
Duloxetine 60 mg			27.4	1.9	26.0	1.9	25.6	1.9	24.6	1.8	26.2	2.0	24.3	1.8
Lacosamide 100 mg			27.1	1.9	30.1	1.9	29.3	1.9	28.7	1.8	31.5	2.1	28.2	1.9
PtP amplitude in capsaicin-irritated skin (μV)														
Placebo	21.8	2.2	23.3	2.8	25.0	2.8	24.8	2.7	22.4	2.7	23.3	2.8	21.6	2.7
Celecoxib 200 mg	19.7	2.1	24.0	2.8	22.2	2.8	22.6	2.7	21.3	2.7	22.0	2.8	20.6	2.7
Pregabalin 150 mg	18.5	1.5	20.2	2.8	21.0	2.8	18.9	2.7	20.3	2.7	19.4	2.7	18.0	2.7
Duloxetine 60 mg	20.7	2.0	22.6	2.8	21.1	2.8	21.0	2.7	21.9	2.7	21.0	2.7	18.7	2.7
Lacosamide 100 mg	20.5	1.7	23.2	2.8	22.9	2.8	23.5	2.7	22.3	2.7	21.1	2.8	21.1	2.7
VAS pain scores in normal skin (mm)														
Placebo	30.3	3.9	35.7	9.1	39.6	9.1	44.1	9.1	45.4	9.2	51.1	9.3	52.6	9.5
Celecoxib 200 mg	34.0	3.7	35.1	9.1	37.7	9.1	40.0	9.1	43.7	9.2	47.1	9.3	47.2	9.5
Pregabalin 150 mg	28.3	3.8	33.1	9.0	32.1	9.1	34.3	9.1	36.0	9.2	36.7	9.3	41.8	9.5
Duloxetine 60 mg	27.4	3.5	34.6	9.0	39.2	9.1	43.2	9.1	44.4	9.2	47.9	9.3	46.0	9.5
Lacosamide 100 mg	28.1	3.2	33.9	9.1	37.4	9.1	44.1	9.1	44.6	9.2	47.6	9.3	48.4	9.5
VAS pain scores in UVB-irradiated skin (mm)														
Placebo			44.0	5.5	49.7	5.3	56.0	5.4	61.25	5.6	65.4	5.9	69.4	5.9
Celecoxib 200 mg			41.1	5.5	42.7	5.3	47.9	5.4	50.53	5.6	56.8	5.9	58.3	5.9
Pregabalin 150 mg			40.2	5.5	42.3	5.3	47.4	5.4	49.35	5.6	54.4	5.9	59.8	5.9
Duloxetine 60 mg			40.3	5.5	47.1	5.3	53.1	5.4	56.85	5.6	61.3	5.9	61.9	5.9
Lacosamide 100 mg			43.4	5.5	50.3	5.3	56.4	5.4	59.22	5.6	66.2	5.9	65.8	5.9
VAS pain scores in capsaicin-irritated skin (mm)														
Placebo	27.7	4.0	42.9	9.3	48.8	9.5	56.0	9.4	59.77	9.5	62.5	9.7	62.2	9.9
Celecoxib 200 mg	26.2	3.8	42.5	9.3	47.4	9.5	53.2	9.4	57.35	9.5	58.9	9.7	59.9	9.9
Pregabalin 150 mg	26.1	3.8	41.3	9.3	43.9	9.5	46.4	9.4	47.40	9.5	52.8	9.7	54.7	9.9
Duloxetine 60 mg	27.3	3.6	41.1	9.3	45.5	9.5	52.2	9.4	57.29	9.5	58.6	9.7	59.7	9.9
Lacosamide 100 mg	20.8	2.8	37.6	9.3	46.4	9.5	53.2	9.4	55.24	9.5	58.2	9.7	59.2	10.0

duloxetine) according to the allocated treatment sequence. The latter subject was included in the safety analysis set but excluded from the per-protocol set (see Figure 2 for progression of study participants).

Effects of placebo and single doses of analgesics on LEP (PtP amplitude) and VAS postlaser pain score in the three different skin conditions

Least square means and mean differences from placebo, and corresponding *P* values for N2–P2 PtP amplitudes of averaged LEP and VAS scores measured over the 6 h period are reported with CIs/standard errors of the mean in Tables 1 and 2, respectively.

The mean overall latencies of the LEP components N2 and P2 were approximately 153 ms for N2 (CI 20.5) and 269 ms for P2 (CI 27.0) after nociceptive stimulus presentation (data not shown). The latencies of the single LEP components (N2, P2) did not allow any significant differentiation of the pharmacological effects between the selected medications and, for example, were not elevated *vs*. placebo.

For all parameters and for all three skin conditions, the overall tests for treatment differences between the five treatment groups demonstrated statistically significant differences, with *P*-values <0.001.

The results for all pair-wise comparisons are shown below.

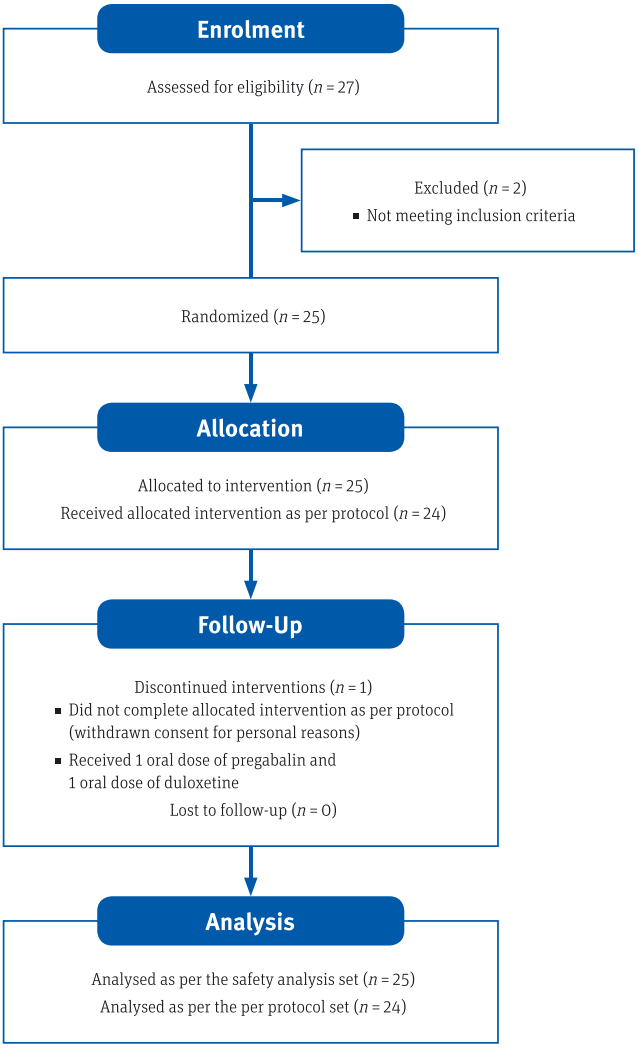

Figure 2

CONsolidated Standards of Reporting Trials (CONSORT) 2010 flow diagram demonstrating the progression of participants through the study

Effect of placebo and single doses of analgesics on LEP (PtP amplitude) and VAS postlaser pain scores in normal skin. Compared with placebo, single doses of duloxetine and pregabalin caused a decrease in the PtP amplitudes of LEPs in normal skin, with an effect starting between 1 h and 2 h postdose and maintained for up to at least 6 h postdose (Figure 3A and Table 2). LEP amplitudes remained unaffected by celecoxib in normal skin. No consistent effect was seen following treatment with lacosamide, although smaller PtP amplitudes were measured 5 h and 6 h after its administration. There only remain significant reductions in the averaged 6 h postdose PtP amplitudes following treatment with duloxetine (−1.7 μV; $P < 0.05$) and pregabalin (−2.68 μV; $P < .001$), when compared with placebo (Table 1).

In agreement with the obtained LEP results, treatment with pregabalin noticeably reduced the VAS score over the 6 h postdose assessment period, compared with placebo (Figure 4A and Table 2). By contrast, the effects of the other analgesics on VAS score time profiles showed little differentiation, visually, from placebo, although VAS scores following the administration of celecoxib were consistently below the values obtained in subjects treated with placebo. Overall, duloxetine and lacosamide failed to reduce the averaged 6 h postdose VAS scores significantly compared with placebo, but significance was achieved with single doses of pregabalin (−9.1 mm; $P < 0.001$) and celecoxib (−2.94 mm; $P < 0.05$) in normal skin (Table 1).

Effect of placebo and single doses of analgesics on LEP (PtP amplitude) and VAS postlaser pain scores in UVB-irradiated/inflamed skin. In contrast to lacosamide, all of the other tested analgesics caused rapid, profound and sustained reductions (up to at least 6 h postdose) in PtP amplitudes of LEPs in UVB-irradiated/inflamed skin (Figure 3B), when compared with placebo. Celecoxib induced the most pronounced mean reduction, followed by duloxetine and pregabalin. Compared with placebo, the averaged 6 h postdose PtP amplitudes were significantly reduced by treatment with celecoxib (−6.20 μV; $P < 0.001$), duloxetine (−4.54 μV; $P < 0.001$) and pregabalin (−3.72 μV; $P < 0.001$) (Table 1).

In line with the LEP results, VAS score profiles obtained after single doses of pregabalin, celecoxib and duloxetine were consistently lower than with placebo (Figure 4B and Table 2), although the reduction induced by duloxetine was less pronounced than for pregabalin and celecoxib. VAS score profiles resulting from treatments with placebo and lacosamide were approximately superimposable. Averaged VAS scores in UVB-irradiated/inflamed skin over the 6 h postdose assessment period were significantly decreased by pregabalin (−8.71 mm; $P < 0.001$), celecoxib (−8.08 mm; $P < 0.001$) and, to a lesser extent, by duloxetine (−4.19 mm; $P < 0.001$) (Table 1).

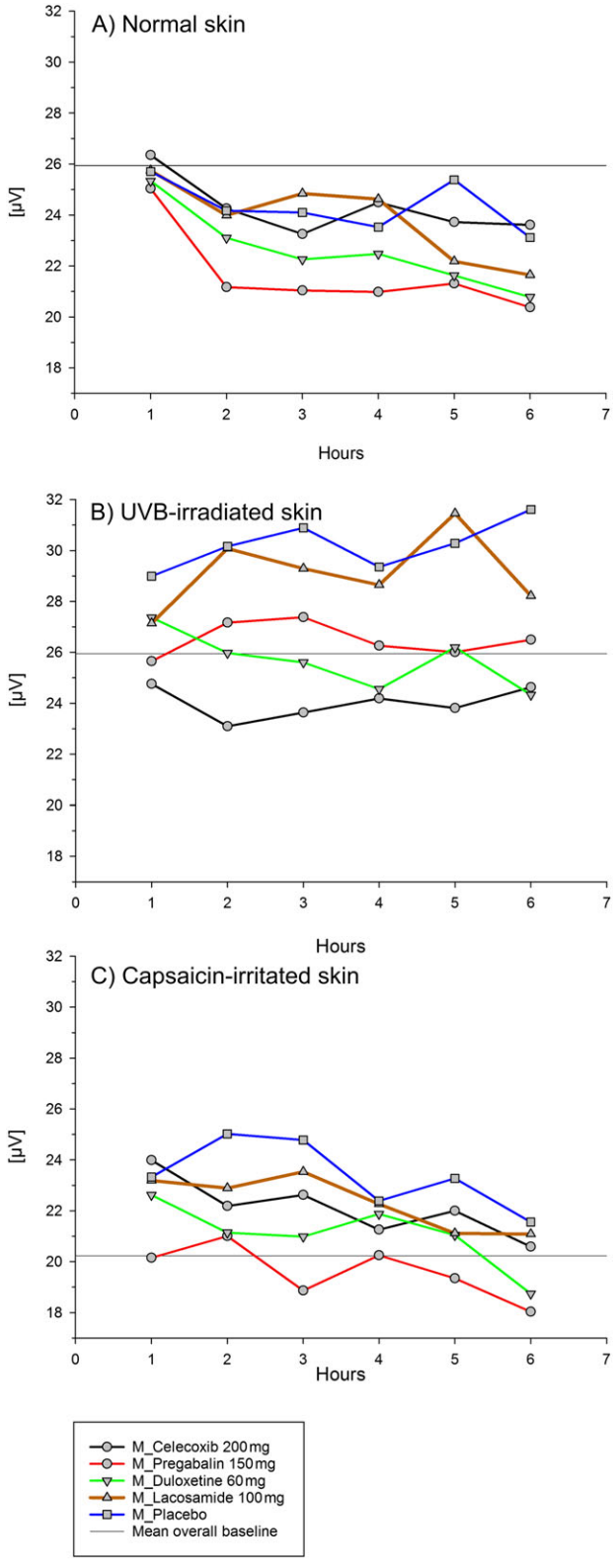

Figure 3

Time course of laser-evoked potential peak-to-peak amplitudes (in µV) from normal skin (A), ultraviolet (UV) B-irradiated skin (B) and capsaicin-irritated skin (C). Least square means from 1 h to 6 h following a single-dose drug administration (n = 24 subjects). The solid horizontal line represents the overall predose baseline

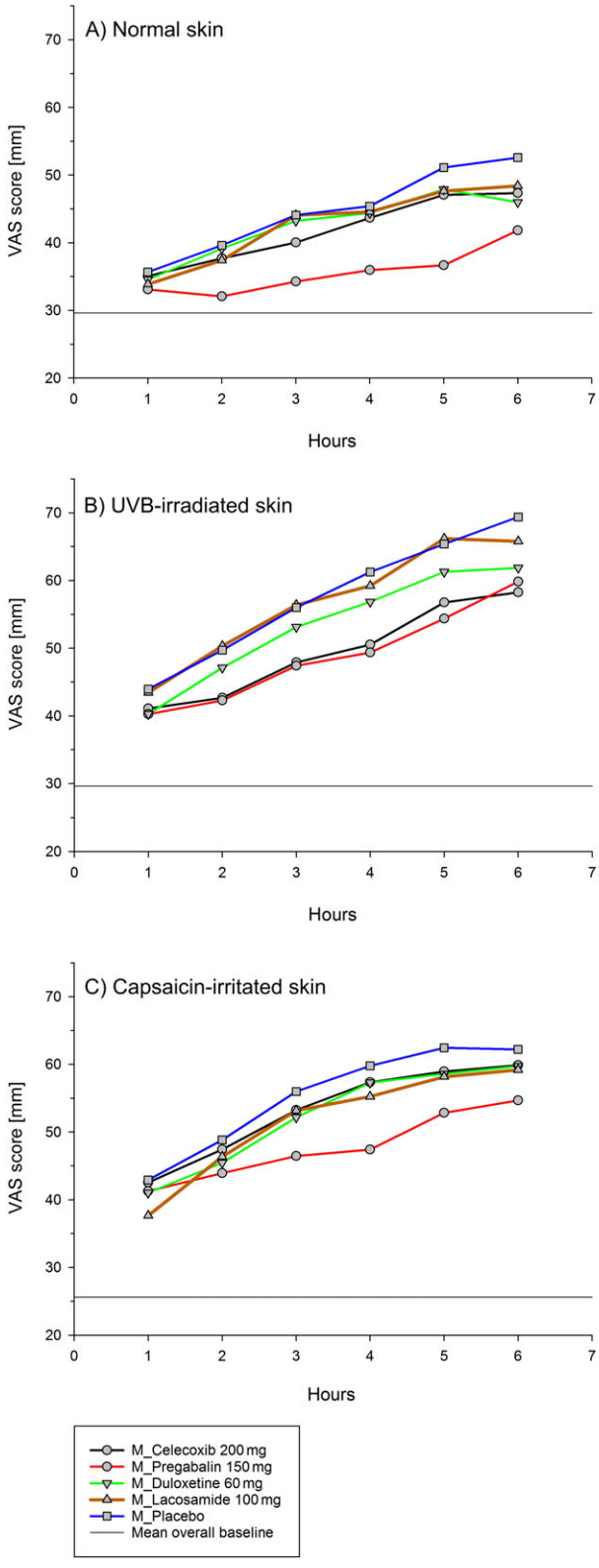

Figure 4

Time course of visual analogue scale (VAS) postlaser pain score (in mm) from normal skin (A), ultraviolet (UV) B-irradiated skin (B) and capsaicin-irritated skin (C). Least square means from 1 h to 6 h following a single-dose drug administration (n = 24 subjects). The solid horizontal line represents the overall predose baseline

Effect of placebo and single doses of analgesics on LEP (PtP amplitude) and VAS postlaser pain scores in capsaicin-irritated skin. PtP amplitudes of LEPs on capsaicin-irritated skin were reduced by treatment with pregabalin and duloxetine, and, to a lesser extent, by celecoxib and lacosamide, when compared with placebo (Figure 3C and Table 2). Treatment with pregabalin showed a markedly greater effect than duloxetine, although both analgesics induced pronounced decreases in PtP amplitudes, which persisted for at least 6 h postdose. This resulted in significant reductions in the averaged PtP amplitudes following treatment with pregabalin (−3.78 μV; $P < 0.001$) and duloxetine (−2.32 μV; $P < 0.05$), but not celecoxib (−1.28 μV; $P = 0.099$) and lacosamide (−1.05 μV; $P = 0.176$), when compared with placebo (Table 1).

Starting at 2–3 h postdose, and regardless of the analgesic treatment administered, the mean VAS (pain) score time courses were lower than after treatment with placebo, although the effect of pregabalin was greater (Figure 4C and Table 2). The VAS score profiles of celecoxib, duloxetine and lacosamide were almost superimposable. Averaged VAS scores in capsaicin-irritated skin over the 6 h postdose assessment period were significantly reduced by a single dose of pregabalin (−7.61 mm; $P < 0.001$) and, to a much lesser, although still significant, extent, by lacosamide (−3.74 mm; $P < 0.05$) and duloxetine (−2.98 mm; $P < 0.05$). Celecoxib also slightly reduced the mean averaged VAS score but this failed to achieve significance (−2.14 mm; $P = 0.085$) (Table 1).

Safety

There were no serious AEs in the present study. A total of 32 AEs, all treatment emergent, were reported by a total of 14 different subjects (not shown). Of these AEs, four were of moderate intensity and 28 of mild intensity. No AEs were reported in subjects under placebo or celecoxib treatment. A total of 12 subjects reported 21 AEs during the duloxetine treatment period, with nausea and drowsiness being the most frequently reported, each with a total of four occurrences, experienced by two subjects. Out of 10 AEs reported during the pregabalin treatment period, diarrhoea, nausea, tiredness and dizziness were the most frequent, each with two events, experienced by one or two subjects. Only one AE – vomiting – was reported following lacosamide administration. There were no clinically significant changes observed in vital signs.

Treatment compliance

Subjects included in the study and treated with analgesics had measurable plasma concentrations of the administered analgesics at all the scheduled time points of measurements (data not shown).

Discussion

The aim of the study was to assess the predictive validity of the LEP–algesimetry procedure further by testing the analgesic effects of four marketed analgesics from different compound classes (celecoxib, pregabalin, duloxetine, lacosamide) in different skin types (normal, UV-irradiated/inflamed, and capsaicin-irritated skin) in healthy human volunteers.

In agreement with previous findings in healthy volunteers [8–11, 15, 23], the present results showed that both the objective LEP PtP amplitude and the subjective VAS self-rating pain score increased and developed over time in subjects treated with placebo and exposed to repeated laser stimuli to UVB-irradiated and capsaicin-irritated dermatomes, but not to normal skin.

The latter suggests that a repeated laser application to normal skin – interstimulus sites are changed after each laser shot – does not induce any sensitization *per se*. Such a phenomenon, known as cutaneous hyperalgesia, has been shown to develop following acute thermal injuries, UV irradiation and local administration of chemicals such as menthol, camphor, mustard oil or capsaicin [7, 8, 22–27].

In general, hyperalgesia is characterized by a time-dependent increase in response to stimuli of constant intensity due to the development of inflammation/sensitization. The hyperalgesia that occurs following an acute skin exposure to UVB or capsaicin has been described as reflecting some elements close to inflammatory pain, without an alteration of central nociceptive processing and/or spinal/central sensitization in neuropathic pain, respectively [22, 24, 28].

Indeed, UVB-induced erythema (sunburn) involves the cyclooxygenase (COX) cascade and the release of a wide range of inflammatory mediators such as prostaglandins, neuropeptides and inflammatory cytokines, as described in inflammatory pain conditions [25, 29–33].

The topical application of capsaicin to healthy human volunteers causes an increase in the sensitivity of C fibres to heat stimuli [8, 13, 14], a reduction in the heat pain threshold and spontaneous burning pain, as in patients with neuropathic pain [28, 34–36].

As observed previously [9], a progressive increase in the VAS score was generally seen over the 6 h experimental period in subjects treated with placebo, regardless of the skin condition. Such a progressive, continuous increase was not seen with the LEP PtP amplitude, which showed different patterns. Although this apparent discrepancy between the objective LEP and the subjective VAS self-assessment pain score cannot be fully explained, it should be kept in mind that the LEPs only reflect components of nociceptive processing, influenced mainly by the intensity of the nociceptive stimulation, whereas the VAS pain score is a composite of pain perception, as well as of cognitive, emotional and vigilance states – that is, many potential confounding factors. For example, the augmenting effect of negative emotions on experimental pain has been already described by several research groups over the last decade [37]. Therefore, it cannot be excluded that negative emotions resulting from repeated exposure to unpleasant laboratory experimental procedures could contribute, at least in part, to the observed time-dependent increase in the VAS pain score in placebo subjects.

Among the different compounds tested in the present study, a single therapeutic oral dose of the selective COX-2 inhibitor ***celecoxib*** was most effective in reducing laser-evoked nociception in UVB-irradiated/inflamed skin, as measured by a reduction in both PtP amplitude and VAS pain score. The lack of effects of celecoxib on the PtP amplitude

and VAS pain score when the laser was applied to normal and capsaicin-irritated skin is in agreement with previous findings showing the lack of analgesic effects of nonsteroidal anti-inflammatory drugs (NSAIDs; e.g. ibuprofen, etoricoxib) in experimental human pain models involving the stimulation of normal and capsaicin-irritated skin [8, 9, 38, 39]. The present results with celecoxib are in accordance with the MoA of NSAIDs, which essentially inhibit the formation of the inflammatory mediator prostaglandin E2 at the site of inflammation. These results are also in line with the proven activity of these drugs in clinical inflammatory pain conditions [40, 41] and in experimental human UVB pain models [8, 9, 25, 39]. The results of the present study further support the potential usefulness of LEPs plus UVB irradiation for identifying novel analgesic compounds with anti-inflammatory properties.

In untreated and capsaicin-irritated skin, a single oral therapeutic dose of ***pregabalin*** induced the most profound reduction in PtP amplitud e and VAS pain score, when compared with other tested compounds and placebo. It also showed a sustained effect on UVB-irradiated skin, although a less pronounced effect on the PtP amplitude in comparison with celecoxib (although a comparable effect on the VAS score). The analgesic activity of pregabalin found here is consistent with its reported large spectrum of clinical analgesic activity via inhibition of nociceptive pathways, including voltage-gated calcium channel mechanisms [42–44], and with its previously observed antinociceptive effects in healthy human subjects in experimental pain models, such as the intradermal capsaicin injection paradigm [45], and for the treatment of fibromyalgia [42, 43]. Of note, although pregabalin does not have anti-inflammatory activity, there is evidence that it attenuates acute postoperative pain [45, 46], which is consistent with its MoA in affecting neuronal transmission in the pain processing pathways, and also the findings obtained in UVB-irradiated skin in the present study. Finally, in line with the proven clinical effects of pregabalin and other anti-epileptic drugs, such as gabapentin and carbamazepine [42, 47], pregabalin induced a consistent antinociceptive effect on objective and subjective readouts, following (laser) heat stimulation on capsaicin-irritated skin, which has been hypothesized to resemble neuropathic pain symptoms [8, 10, 28].

Duloxetine, a serotonin norepinephrine reuptake inhibitor marketed for the treatment of neuropathic pain, exerts its analgesic activities via descending inhibition in pain signal processing by 5-hydroxytryptamine and norepinephrine mechanisms [48]. In line with this nonspecific mod ulatory activity of the pain pathway, duloxetine administered as a single oral d ose resulted in consistent analgesic activity in normal, UVB-irradiated and capsaicin-irritated skin types. The observed objective and subjective effects were, in general, less pronounced than after administration of pregabalin, except for the mean reduction in PtP amplitude obtained after laser stimulation of UVB-irradiated skin. Although the numerical relevance of such a difference in the present experimental human model cannot be easily explained after a single dose administration in an unpowered study, the fact that duloxetine, in contrast to pregabalin, has demonstrated unequivocal analgesic effects in several randomized controlled studies conducted in patients with osteoarthritis of the knee [48–51] suggests that it may be more potent than pregabalin in attenuating pain in inflammatory conditions [52].

In agreement with the inconclusive results from clinical efficacy studies with ***lacosamide***, with its slow inactivation of (unspecific) voltage-gated sodium channels, for the treatment of diabetic neuropathy and other pain conditions [52], lacosamide failed to demonstrate any consistent analge-sic effect in the present study.

In conclusion, single oral therapeutic doses of pregabalin, duloxetine and celecoxib, but not lacosamide, showed rapid (between 1 h and 2 h) and sustained (>6 h) antinociceptive/antihyperalgesic effects in this experimental, objective, quantitative human algesimetric model. These results are in line with the known clinical profiles of these pain medications and confirm that the present experimental paradigm in healthy volunteers is pharmacologically sensitive for assessing analgesics with different MoAs. The present study complements previous trials, in which a different list of analgesics was tested in comparable experimental conditions [8, 9, 22].

The previous and present results strongly suggest that the objective LEP–algesimetry procedure may be useful when implemented in ECD [17]. It may be used to assess the effects of novel potential analgesics and thereby support early go/no-go decisions before they enter into long and costly phase II trials in patients. Furthermore, this paradigm could be useful for determining the most promising dose range [9, 15] and the most suitable dosing schedule, and hence support the design of subsequent clinical studies. Finally, as shown by the differential effects of the tested compounds across the diverse skin types, reflecting different pain conditions, this test paradigm could be useful for determining the best target patient population (e.g. nociceptive/inflammatory *vs.* neuropathic pain) for a compound with a specific MoA.

In summary, the objective LEP–algesimetry procedure, in combination with different skin types, is a promising tool for supporting the ECD of new analgesics. Additional studies are now warranted to assess whether the inhibitory effects of analgesic agents with new MoAs on PtP amplitude and VAS scores measured during ECD translate into efficacy in subsequent patient trials.

Competing Interests

There are no competing interests to declare.

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