Effects of target-controlled infusion of high-dose naloxone on pain and hyperalgesia in a human thermal injury model: a study protocol

A randomized, double-blind, placebo-controlled, crossover trial with an enriched design

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Abstract

Mu-opioid-receptor antagonists have been extensively studied in experimental research as pharmacological tools uncovering mechanisms of pain modulation by the endogenous opioid system. In rodents, administration of high doses of mu-opioid-receptor antagonists after the resolution of an inflammatory injury has demonstrated reinstatement of nociceptive hypersensitivity indicating unmasking of latent sensitization. In a recent human study, pain hypersensitivity assessed as secondary hyperalgesia area (SHA), was reinstated 7 days after a mild thermal injury, in 4 out of 12 subjects after a naloxone infusion.

The aims of the present study are first, to replicate our previous findings in a larger-sized study; second, to examine if high sensitizers (subjects presenting with large SHA after a thermal injury) develop a higher degree of hypersensitivity after naloxone challenge than low sensitizers (subjects presenting with restricted SHA after a thermal injury); and third to examine a dose–response relationship between 3 stable naloxone concentrations controlled by target-controlled infusion, and the unmasking of latent sensitization.

Healthy participants (n=80) underwent a screening day (day 0) with induction of a thermal skin injury (47°C, 420 seconds, 12.5 cm²). Assessment of SHA was performed 1 and 2 hours after the injury. Using an enriched design, only participants belonging to the upper quartile of SHA (Q4, high sensitizers; n=20) and the lower quartile of SHA (Q1, low sensitizers; n=20) continued the study, comprising 4 consecutive days—days 1 to 4. Thermal skin injuries were repeated on day 1 and day 3, whereas day 2 and day 4 (7 days after day 1 and day 3, respectively) were target-controlled infusion days in which the subjects were randomly allocated to receive either naloxone (3.25 mg/kg, 4 mg/mL) or placebo (normal saline) intravenous. The primary outcome was SHA assessed by weighted-pin instrument (128 mN) 0, 1, 2, and 165 to 169 hours after the thermal injury (day 1–4). The secondary outcomes were pin-prick pain thresholds assessed by weighted-pin instrument (8–512 mN) at primary and secondary hyperalgesia areas (days 1–4).

The naloxone-induced unmasking of latent sensitization is an interesting model for exploring the transition from acute to chronic pain. The results from the present study may provide valuable information regarding future research in persistent postsurgical pain states.

Abbreviations: LS = latent sensitization, MOR = mu-opioid-receptor, PPT = pin-prick pain threshold, SHA = secondary hyperalgesia area, TCI = target-controlled infusion, WPI = weighted-pin instrument.

Keywords: enriched design, humans, latent sensitization, naloxone, randomized controlled trial, secondary hyperalgesia, thermal injury
1. Introduction

Endogenous opioid analgesia could be impaired or altered in chronic pain conditions,[1,–4] and this may play an important role in the transition of acute to chronic pain in humans.[5–7] Naloxone and naltrexone are mu-opioid-receptor (MOR) antagonists and inverse agonists, often used in experimental research to determine the contribution of endogenous opioids to the central processing of pain.[8,9] Naloxone-dose-dependently produces either hypoalgesic or hyperalgesic properties.[10] Recent high-profile studies in rodents indicate that endogenous MOR analgesia masks latent sensitization (LS).[11,12] LS is a silent form of central sensitization, defined as an increased responsiveness of central nervous system (CNS) nociceptive neurons to afferent input after tissue injury. LS persists beyond tissue healing and resolution of hyperalgesia. When MOR antagonists are administered during this resolution phase, we and others have observed a reinstatement of hypersensitivity to a somato-sensory stimulus, which we interpret as the unmasking of LS.[1,5,6,11,12]

Using a randomized, controlled, crossover study design, we initially reported that an intravenous naloxone dose of 21 μg/kg, delivered 7 days after a mild heat injury, failed to produce reinstatement of hyperalgesia.[13] In a follow-up study, however, we found that a higher intravenous dose of 2.0 mg/kg did produce reinstatement of hyperalgesia in 4 out of 12 subjects.[14] The naloxone-induced hyperalgesia area extended beyond the primary heat injury area and was therefore termed secondary hyperalgesia. Compared with the 8 nonresponders, the 4 responders developed larger secondary hyperalgesia areas (SHAs) in the hours immediately after exposure to the heat injury.

The current study examines 3 important questions. First, are the results of the 2.0 mg/kg study replicable with a greater number of subjects? Second, is it possible to predict which individuals will express naloxone-induced reinstatement of hyperalgesia? Third, is naloxone-induced unmasking of LS a dose-dependent process?

The aims of the present study thus are to: replicate and validate our previous findings; determine whether “high sensitizers” express larger hyperalgesia areas after naloxone challenge than “low sensitizers”, using an enriched design; and examine if the unmasking process depends on the naloxone concentration, using a target-controlled infusion (TCI) technique.

2. Methods

2.1. Study management

The study was conducted in accordance with the guidelines and rules concerning quality control and quality management on clinical trials involving humans, and followed the Good Clinical Practice and the Good Manufacturing Practice guidelines. All data about potential and enrolled participants were treated confidentially. Only the investigators and relevant authorities had access to the data and eventual amendments to the protocol were communicated to the relevant authorities, and the entries on the registry databases were updated. The study was approved by the Committee of Health Research Ethics of the Capital Region (RH-15018869), Danish Medicines Agency (2015-003426-19), and the Data Inspection Authority of the Capital Region (RH-2015-284, I-suite nr. 04296). The study is registered in EUDRACT (2015-003426-19) and in ClinicalTrials.gov (NCT02684669). The approved final protocol version was: Protocol-High- NxTME_v1.5_13.01.2016.

2.2. Participants

The participants were recruited from a registry of participants previously participating in experimental pain studies at the Multidisciplinary Pain Center 7612, Neuroscience Center, Copenhagen University Hospitals (ADS). The participants had specifically given their verbal or written consent that they may be contacted in future research. If the investigators experienced difficulty in recruitment of participants, an advertisement was placed in a Danish e-site for recruitment of participants in medical research (forsoegsperson.dk). Additionally, the advertisement was posted on facebook.com and hung up on bulletin boards at the University of Copenhagen.

The participants received written information about the study by the investigator (ADS). The investigator then scheduled a physical meeting with the participant, informing the participant of the right to bring a relative or a friend for the information meeting. At the meeting, taking place in an undisturbed environment, the investigator gave verbal information concerning the study and answered the participant’s and the companion’s questions. The participant was informed that he had the time to consider participation in the study. Two days after the meeting, the investigator called or sent an e-mail, making an appointment with the participant for the study day.

On the study day, the investigator repeated the verbal information and answered any questions the participant had. The written consent was obtained on the study day before any assessments were done. If the participant needed more time before signing the informed consent, a new study day was scheduled. The participant was informed that the participation in the study was voluntary and that he may withdraw from the study at any time without further notice or explanation. This action will not have any consequence for ongoing or future medical treatments at the involved departments or hospitals. Also, the participant was informed that the investigator may exclude the participant from the study at any time. Participants received an hourly payment for attending the study of DKK 150 (USD 25), and, if required by the participant, reimbursement for transportation costs.

2.3. Laboratory environment

The experimental procedures took place at the Multidisciplinary Pain Center in a quiet, well-lit room (22–25°C, relative humidity (RH) 20%–45%). Participants adopted a relaxed recumbent position during sensory assessments, but were otherwise allowed to ambulate between assessments. However, during and immediately after infusion procedures, participants were asked to adopt a comfortable, recumbent position.

2.4. Study design

A randomized, placebo-controlled, double-blind, crossover design was used. The study included 1 screening day (day 0) and 4 experimental days (days 1–4; Fig. 1). Since day 0 was for selecting the lower quartile (Q1, low sensitizers) and the upper quartile (Q4, high sensitizers) of the participants, regarding the area of secondary hyperalgesia developed after a thermal (heat) injury,[15,16] an enriched design was used. Only participants belonging to the Q1 or Q4 quartile were included in days 1 to 4.

2.5. Randomization

2.5.1. Low versus high sensitizers. After day 0 (n = 80), that is, the selection into low sensitizers (Q1, n = 20) and high sensitizers (Q4, n = 20), a random permutation of numbers from 1 to 40 was applied (randomization.com). Each low and high sensitizer received a sequential rank order from 1 to 40 according to the magnitude of the SHA. These sequential rank numbers were then
consecutively paired with the list of a random permutation of numbers, rendering a randomization of the order of low and high sensitizers. This randomization procedure was performed by the senior author (MUW; not participating in the assessments), blinding the examiner to the participant’s Q1 or Q4 affiliation.

2.5.2. Drug allocation. Computer-generated sequence randomization, using blocks of 4 subjects (randomization.com), was performed by Skanderborg Hospital Pharmacy, also responsible for manufacturing, packaging, and labeling of the drugs. Two sets of nontransparent sealed envelopes, 1 for each participant, were prepared. The envelope contained information on treatment allocation order. The sponsor (MUW) kept one of the sets of envelopes stored, whereas the other set was kept by the investigators (ADS, EKJ). Both sets of envelopes were kept in separate, securely locked, prespecified filing cabinets. Additionally, each drug package included a sealed, nontransparent envelope containing the appropriate randomization sequence code, to be broken in case of a medical emergency. The participants and the investigators were blinded to the treatment code, to be broken in case of a medical emergency. The participants were then asked to fill out psychometric questionnaires, and to complete an online reaction time test (for details see below). Finally, before inclusion, a simple drug-screening test for opioids was made. After a brief medical examination by the investigator, the Clinical Opiate Withdrawal Scale (COWS) was completed. Participants were then asked to sign an informed consent, meeting the inclusion criteria and affirming the exclusion criteria (Table 1; ADS, MUW). After a brief medical examination by the investigator, the Clinical Opiate Withdrawal Scale (COWS) was completed. Participants were then asked to fill out psychometric questionnaires, and to complete an online reaction time test (for details see below). Finally, before inclusion, a simple drug-screening test for opioids was made. After inclusion, induction of a thermal skin injury was performed, and SHAs were assessed (Fig. 2).

As previously presented, further participation depended on the magnitude of the SHAs developed at day 0: participants belonging to Q1 or Q4 continued through days 1 to 4, whereas those belonging to Q2 and Q3 were not included in further analyses.

2.6. Study setup

2.6.1. Day 0. A general algorithm of the study is presented in Fig. 1. On day 0, participants were asked to sign an informed consent, meeting the inclusion criteria and affirming the exclusion criteria (Table 1; ADS, MUW). After a brief medical examination by the investigator, the Clinical Opiate Withdrawal Scale (COWS) was completed. Participants were then asked to fill out psychometric questionnaires, and to complete an online reaction time test (for details see below). Finally, before inclusion, a simple drug-screening test for opioids was made. After inclusion, induction of a thermal skin injury was performed, and SHAs were assessed (Fig. 2).

As previously presented, further participation depended on the magnitude of the SHAs developed at day 0: participants belonging to Q1 or Q4 continued through days 1 to 4, whereas those belonging to Q2 and Q3 were not included in further analyses.

2.6.2. Days 1 to 4. On days 1 and 3, thermal injuries were induced, assessing SHAs (Figs. 1 and 2). On days 2 and 4, TCIs of naloxone or placebo were administered exactly 7 days after induction of the thermal injury (Fig. 2). Before the examinations at day 2 and day 4, the drug-screening tests for opioids were reiterated.

2.7. Intervention

2.7.1. Target-controlled infusions. The 3-step TCI algorithm has previously been reported. The algorithm based on pharmacokinetic data from the study by Dowling et al[17] was calculated by the software NONMEM [7.3 ICON Development Solutions, Manchester, UK (property of UCSF, US)], using computer simulations based on a population-kinetic model with 2000 simulated administrations distributed on 10 subjects. The estimated mean (10% and 90% percentiles) plasma concentrations of naloxone at each TCI step are illustrated in Fig. 3. A total dose of 3.25 mg/kg of naloxone (4 mg/mL) vis-a-vis 0.81 mL/kg of placebo (normal saline) was administered in a stepwise approach (Table 2). Each step contained a 1-minute bolus and a 24-minute infusion. During the last 10 minutes of each step, plasma concentrations were considered to be stable, and therefore the pharmacodynamic assessments were performed (Fig. 3). The infusion was to be discontinued at any step if the participant’s pain ratings at rest ≥5, assessed by the numerical rating scale (NRS: 0 = no pain, 10 = worst possible pain imaginable).

Two syringes (BD Perfusion syringe: Becton, Dickinson and Company Limited, Louth, Ireland) were each be filled with a 50-mL solution of either naloxone or placebo and inserted into a syringe-based pump (Perfusor Space Infusion System 8713030: B. Braun Melsungen AG, Melsungen, Germany).

2.7.2. Rescue drugs. Epinephrine 1 mg/mL, and other relevant drugs and equipment for cardiopulmonary resuscitation were
available. An anesthesiologist (MUW) on call, dedicated to the study, resided in a room adjacent to the laboratory and was instantly available at all times during the study. Midazolam 1mg/mL and alfentanil 0.5mg/mL were available for management of agitation and anxiety, and for the treatment of severe acute pain, respectively. Other drugs that the investigator considered necessary for the management of adverse effects (atropine, ondansetron) were administered as needed. The administration of rescue drugs was recorded in the participant’s Case Report Form.

2.8. Monitoring

On TCI days (days 2 and 4), the participants were continuously monitored with 3-lead ECG, heart rate, and pulse oximetry, and noninvasive blood pressure and respiratory rate were regularly assessed. Monitoring started before infusion and ended 1 hour 40 minutes after the start of infusion. Other signs of adverse effects, for example, epigastric pain, headache, lethargy, nausea, and photophobia, were reported in the Case Report Form.

2.9. Questionnaires

2.9.1. Clinical Opiate Withdrawal Scale. The COWS is a an examiner-based scale evaluating signs of opioid withdrawal.[19] Quantitative assessments of heart rate changes, diaphoresis, restlessness, pupil size, bone or joint aches, running nose or tearing, nausea, vomiting, diarrhea, tremor, yawning, anxiety or irritability, and “goose-flesh” were categorized into 11 categories. COWS scores were graded as follows: 5 to 12 = mild; 13 to 24 = moderate; 25 to 36 = moderately severe; >36 = severe.

2.9.2. The Hospital Anxiety and Depression Scale. The Hospital Anxiety and Depression Scale (HADS) evaluates anxiety and signs of depression[20,21] based on 14 questions about the subject’s status in the previous week (each subscale contains 7 questions). Participants rated each question on a scale of 0 to 3. The 2 subscales were summed separately. The maximum score on each subscale was 21 points, and a score of 11 or more points suggested that the participant likely or definitely was suffering from anxiety or depression. In the case of score >11 points in the depression subscale of the HADS, a physician decided if there are clinical signs of depression. If there were signs of depression, the diagnosis was disclosed to the participant. The participant was informed that the diagnosis of depression was based on clinical judgment (the HADS scale can be included in the diagnostic procedure). If it is the participant’s wish, he should visit his general practitioner for diagnosis and eventual treatment. Participants with significant signs of depression were excluded from the study.

2.9.3. The Pain Catastrophizing Scale. The Pain Catastrophizing Scale (PCS) consists of 13 questions divided into 3 sections: rumination, exaggeration, and helplessness.[22] The answers were rated on an ordinal scale (0 to 4 points) and summed. Sign of pain catastrophizing was a total score >30 points.

Figure 2. Day 1/day 3 (cf. Fig. 1) includes baseline assessments (green rectangle, 0 minute), induction of a thermal injury (TI, red rectangle, 20 minutes), and postinjury assessments (blue rectangles: 1 hour 27 minutes and 2 hours 27 minutes). Day 2/day 4 (cf. Fig. 1) includes a predrug assessment (magenta rectangle; postinjury 165 minutes), drug infusions (naloxone or placebo; 167 hours 35 minutes; 168 hours 0 minute; 168 hours 25 minutes) and assessments during target-controlled infusion (TCI; grey rectangles). The estimated TCI plasma concentrations are superimposed in dashed red line. Numbers (1–3) during the infusion period indicate the 3 TCI steps. Timelines for assessments of pin-prick pain thresholds are indicated by red stars, and secondary hyperalgesia areas by green stars.

Figure 3. Test algorithm day 2/day 4 with superimposed naloxone plasma–concentration curves. Median plasma concentration (red) with 10% and 90% percentiles ranged (dashed black lines) during a 3-step target-controlled infusion (TCI). Naloxone is given at step 1 (S1: 15–25 minutes) with 0.25 mg/kg, step 2 (S2: 39–49 minutes) 0.75 mg/kg, and step 3 (S3: 65–75 minutes) 2.25 mg/kg. Yellow timelines represent ratings with Clinical Opiate Withdrawal Scale (COWS) and blue columns indicate sensory testing (BL and S1–3: secondary hyperalgesia areas; BL and S3: pin-prick pain thresholds). BL = baseline assessments (reproduced with permission from trials).[18]
2.10. Sensory assessments

2.10.1. Online reaction time. Online reaction time was based on a free-ware computer application showing a red-green traffic light.[23,24] Participants were instructed to press the button when the light changed from red to green, and the reaction time in milliseconds was indicated. Three measurements were obtained, and the median value was used as a representative estimate of reaction time.

2.10.2. Thermal injury. An area 2.5 × 5.0 cm² with the longitudinal (large) axis pointing cephalad was outlined by a marker on the medial aspect of the lower leg with the upper margin 11 cm from the anterior margin of the tibia.[13] The participants were instructed to use a hair trimmer in the assessment area 2 days before the study, to avoid any interference with the sensory assessments. In case of inadequate trimming, the investigator removed any hair in the assessment area using a surgical hair trimmer (Surgical Clipper 9691, 3M Healthcare, MN). Day 0, day 1, and day 3 first-degree thermal injuries were induced in the area by a computerized contact thermode (MSA Thermal Stimulator, Somedic AB, Horby, Sweden; 5.0 × 2.5 cm²; baseline: 32°C; ramp rate: + 1°C/s; plateau: 47°C; duration: 420 seconds) applied with gentle pressure (as previously described).[24,25] During the thermal injury, pain assessments (NRS) were made at 0, 30, 60, 120, 180, 240, 300, 360, and 420 seconds.

2.10.3. Assessment of secondary hyperalgesia areas. Areas of secondary hyperalgesia were assessed by a weighted-pin instrument (WPI; 128 μmN corresponding to 2.606 kPa; MRC Systems, Heidelberg, Germany)[24] delivering punctate stimuli (stimulus area: 0.049 mm²) on day 0, day 1, and day 3: before, and 1 and 2 hours after the thermal injury (Figs. 1–2); and day 2 and day 4: 165 to 169 hours after the thermal injury (baseline: 165 hours 0 minute; during TCI: 167 hours 35 minutes, 168 hours 25 minutes (step 3 of TCI)). The borders of the SHAs were determined by stimulating along 8 symmetric lines converging towards the center of the thermal injury. The punctate stimulations started in normal skin outside the area of secondary hyperalgesia and continued inwards towards the thermal injury area, applying a velocity of 0.5 cm/s and a stimulation rate of 0.7 to 1.0 Hz. The participant was asked, with eyes closed, to report when the punctate sensation changed from an innocuous pin-prick to a stinging, smarting, or unpleasant sensation. When the change was reported, the weighted pin was moved 1 step further in the inward trajectory and the participant had to confirm that the change persisted. If the change was confirmed, the location of the first punctate stimulus was marked on the skin, indicating 1 corner of the delineated octagon. If not confirmed, the punctate stimulation trajectory continued until

perceptual changes in 2 consecutive punctate stimuli were obtained. The secondary hyperalgesia markings were transferred to a clear acetate sheet, and the octagon area was calculated by planimetry using a vector-based drawing program (Canvas 12.0, ACD Systems International, BC, Canada).

If SHAs were delineated at baselines (day 0, day 1, or day 3) or 165 hours after injury (Fig. 2; day 2 or day 4), the participants were asked to compare these perceptions to postinjury sensations (day 0, day 1, or day 3) or sensations during TCI (day 2 or day 4), in regard to quality (stinging, smarting, or unpleasant sensation) and intensity. If the sensations were more stinging, smarting, or unpleasant, and of a higher intensity in the postinjury phase, then the baseline and postinjury 165-hour sensations were not considered to reflect genuine SHAs. If no perceptual difference was registered, the baseline and postinjury 165-hour assessments were subtracted from the area measured after the thermal injury and during the TCI regimen, respectively.

2.10.4. Pin-prick pain thresholds. Pin-prick pain thresholds (PPTs) were assessed by the WPIs (8, 16, 32, 64, 128, 256, 512 mN) using a modified Dixon procedure, both in the primary thermal injury area and in the SHA.[26,27] (Fig. 2). PPTs were assessed on day 0/day 1/day 3 (baseline, 1, and 2 hours postinjury) and day 2/day 4 [postinjury: 165 hours 0 minute and 168 hours 25 minutes (step 3 of TCI)]. Assessment of PPT was not performed during the first and second step of the TCI, due to the risk of development of sensitization, possibly confounding study results. All assessments were performed while the participant had closed eyes. The WPIs were applied each, 5 times perpendicularly to the skin, at a rate of 0.7 to 1.0 Hz, using a randomized distribution pattern, first in the primary injury area and then in the SHA. If no SHA was present, the punctate stimuli were applied in an area 5 cm outside the perimeter of the primary injury area. The WPI with the lowest punctate force yielding 3 or more stinging, smarting, or unpleasant sensations indicated the threshold. An ordinal scale was used (1 = 8 mN, 2 = 16 mN, 3 = 32 mN, 4 = 64 mN, 5 = 128 mN, 6 = 256 mN, 7 = 512 mN, 8 = > 512 mN). The median value of the 5 assessments indicated the PPT.

Table 2

| Step   | TCI           | Time, min | Dose, mg/kg |
|--------|---------------|-----------|-------------|
| Step 1 | Bolus 1       | 0-1       | 0.02        |
|        | Infusion 1    | 1-25      | 0.23        |
| Step 2 | Bolus 2       | 25-26     | 0.06        |
|        | Infusion 2    | 26-50     | 0.69        |
| Step 3 | Bolus 3       | 50-51     | 0.18        |
|        | Infusion 3    | 51-75     | 2.07        |
| Total  |              | 75        | 3.25        |

Target-controlled infusion (TCI) of naloxone (4 mg/mL): timeline and dose/kg for the 3 steps: step 1 (bolus 1 + infusion 1); step 2 (bolus 2 + infusion 2); step 3 (bolus 3 + infusion 3).

2.11. Outcome

2.11.1. Primary outcome. The primary outcome was SHAs assessed by a WPI (128 μmN) 0, 1, 2, and 165 to 169 hours after the thermal injury (days 1–4).

2.11.2. Secondary outcomes. The secondary outcomes were PPTs assessed by WPIs (8–512 mN) at primary and SHAs (days 1–4).

2.11.3. Tertiary outcomes. Tertiary outcomes were pain ratings (NRS) during thermal injury (day 1 and day 3), HADS (day 0), PCS (day 0), COWS (day 2 and day 4), online reaction time (days 1–4).

2.12. Sample size calculation

Data from our previous high-dose naloxone study[14] in high sensitizers (n = 3; Q4), 168 hours after the thermal injury, indicated mean (SD) values of SHAs during placebo infusion of 2.1 cm² (2.5 cm²), and during naloxone infusion 111.0 cm² (26.3 cm²). Corresponding data for the low sensitizers (n = 3; Q1) were during placebo infusion 0.3 cm² (0.1 cm²) and during naloxone infusion 0.9 cm² (0.6 cm²). Using a significance level of 0.01 (α) and a power of 0.90 (β = 0.10), the estimated number of
individuals needed to reject the null hypothesis in the high sensitizers were 5 (effect size 4.3) and in the low sensitizers were 18 (effect size 1.1) (G*Power3.9.1.2, Kiel University, Germany). However, because the data used for sample size estimates were provided by an extremely limited number of individuals, and, because the nature of the study also was proof of concept, it was decided to include 20 high sensitizers and 20 low sensitizers (as previously mentioned using an enriched design, based on a screening day 0 of 80 subjects). Boot-strapping techniques could have been used, but we considered the number of individuals used in the sample to estimate too extreme. On the other hand, if a larger number of subjects were needed, the clinical significance was deemed of dubious value.

2.13. Statistics
To test if data were normally distributed, residual plots and the Kolmogorov–Smirnov test were used (SPSS IBM Software 22.0, Chicago, IL; MedCalc Software; version 16.4.3, Mariakerke, Belgium). In the case of non-normal data distribution, an attempt for normalization of data was applied, either using logarithmic transformation or a Box-Cox transformation.[23]

A mixed model with random effect for subject and fixed effects for the variables sensitizers (high sensitizers/low sensitizers), intervention (naloxone/placebo), TCI (step 1/step 2/step 3), and PCS scores were used for the primary outcome secondary hyperalgesia. Nonsignificant (P>0.05) factors, beginning with interactions, were excluded until all included factors attained significance. Main effects and interaction effects were examined.

For sake of clarity, conventional comparisons of SHAs, using simple calculation of the principal differences, are shown below. Statistics were by paired and unpaired t-tests, or Mann–Whitney and Wilcoxon tests, depending on data distribution.

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\Delta SHA = SHA_{Q4} - SHA_{Q1}
\]

where SHA = secondary hyperalgesia areas; Q1 = belonging to the lower quartile; Q4 = belonging to the upper quartile; NX = naloxone; and PL = placebo. The maximal SHA values during the TCI (irrespective of the TCI step) are used.

The risks of type I (α) and type II (β) errors were set to 0.01 and 0.10, respectively. Statistical calculations were performed initially with partially unblinded data, that is, groups A and B. When these statistical calculations had been completed, treatment allocations were fully unblinded into naloxone and placebo groups. Intention-to-treat (ITT) and per-protocol (PP) data were analyzed separately.

3. Safety issues
3.1. Thermal injury
The first-degree thermal injury is a validated pain model.[29] The induction of the injury is a moderately painful procedure, but not associated with spontaneous pain after termination of the heat stimulus. Morphologically, the injury is comparable to a slight thermal injury. The induction of the injury is a moderately painful procedure, but not associated with spontaneous pain after termination of the heat stimulus. Morphologically, the injury is comparable to a slight thermal injury.

If the participant experiences severe pain during induction of the thermal injury, the participant is able to discontinue the heating by pushing a button terminating the stimulus or by saying so to the investigator. Occurrence of a more severe second to third-degree thermal injury has been described following the use of a malfunctioning, overheating contact thermode. Due to the rigorous, compulsory testing paradigms of our thermodes, we have not experienced any thermal adverse effects during the past 5 years with the thermal injury model (H-2-2010-115; H-2-2012-036; H-2-2012-174; H-1-2013-045; H-4-2013-013), including more than 500 experimental thermal injuries.

3.2. Development of a sustained pain state due to the high-dose naloxone
With regard to the risk of development of a sustained or even persistent pain state after the naloxone administration after the thermal injury, the authors considered it highly unlikely. First, the thermal injury results in a transient pain perception. Second, naloxone does not affect the production of endogenous opioids, but only acts as a short-acting reversible antagonist of opioids. Third, in adults, the elimination (T½β) half-life of naloxone is 54 to 64 minutes.[30,31] Naloxone has no known long-acting metabolites and thus there are no pharmacokinetic reasons for a prolonged reversal of endogenous opioids. Fourth, in rodent studies, administration of MOR antagonists has caused transient episodes of hypersensitivity with a duration of 60 to 90 minutes.[11] Reiterated administration of naltrexone, over the course of months, has confirmed full reversibility of the hypersensitivity episodes. Fifth, in our recent study with intravenous (i.v.) administration of naloxone 2.0mg/kg, very short-lasting changes in hypersensitivity were seen in 4 out of the 12 individuals responding to naloxone.[14] Thus, the authors consider the occurrence of sustained pain to be highly unlikely in this study.

3.3. Naloxone dose considerations
The current study used a naloxone dose of 3.25 mg/kg, which is 600 to 6000 times higher than clinical doses used in the treatment of a severe opioid overdose.[32] Furthermore, a positron emission tomography (PET) study from 1989 indicated that administration of 0.1mg/kg naloxone resulted in complete inhibition of [11C]-carfentanil binding to MOR in humans.[33] Thus, we use a naloxone dose that is approximately 33 times the dose required to occupy all MORs.

The dose selection was determined with two rationales in mind. First, over the course of our previous study to back-translate the human thermal injury model to a rodent model, it was revealed that doses of 3.0 to 10.0 mg/kg of naloxone, delivered 21 days after heat injury to the skin of the plantar hindpaw, were required to precipitate reinstatement of hypersensitivity.[11] Second, in our previous human studies in the heat injury model, low-dose naloxone infusion (21 μg/kg) [33,34] did not reinstate secondary hyperalgesia, whereas high-dose naloxone infusion (2.0 mg/kg) did precipitate reinstatement in 4 out of 12 subjects.[14]

Regarding the toxicity profile, previous studies have shown that systemic doses up to 6.0 mg/kg have been tolerated well in healthy participants,[34–39] and even in higher doses in patients[40–45] with none or only mild to moderate transient adverse effects. However, no methodical evaluation of adverse effects has been performed in these studies. In our previous study
including 3 pilot participants and 12 study participants, 6 subjects reported mild transient adverse effects, including tiredness, epigastric pain, frontal headache, and photophobia. Furthermore, naloxone did not induce any changes in ECG, blood pressure, heart rate, respiratory rate, or oxygen saturation during the experimental days.

In the present study, the unmasking of LS is to be generated with TCI, acquiring 3 successively higher plasma concentration levels of naloxone, a precautionary measure that may reveal adverse effects necessitating discontinuation of the infusion at an early stage. Additionally, the TCI technique may confirm a pharmacodynamic dose–response relationship for LS. In conclusion, the authors, therefore, consider it safe to administer a naloxone dose of 3.25 mg/kg in the present study.

4. Implications of the study

Provided that the study outcome replicates our initial findings, it will highlight several important pathophysiological issues. Extensive tissue injury sensitizes CNS neurons by increasing excitability and synaptic efficiency.[16,17] A prominent manifestation of central sensitization is development of secondary hyperalgesia, where innocuous repetitive or dynamic stimuli in normal tissue surrounding the injured area lead to the triggering of a nociceptive response. Previous reports indicate that MOR antagonists can reinstate hyperalgesia after the resolution of the injured area, suggesting that central sensitization may reside in a silent form, known as LS.[11,12] Although it is generally believed that central sensitization is implicated in a number of chronic pain states,[46] it has been hypothesized that unmasking of LS is a principal mechanism driving the transition from acute to the chronic pain state.[16,11] The current protocol is designed to provide proof of concept for LS in humans in an experimental pain model. This study is a significant step forward to determine the manifestation of LS in clinical postsurgical models, for example, groin hernia repair or dental extraction.[48] Severe persistent postsurgical pain affects 2% to 10% of patients depending on the type of surgery, surgical technique, and patient-related factors, and is a huge and daunting problem for the individual and the society.[49,50] The suggested technique, and patient-related factors, and is a huge and daunting problem for the individual and the society.

In a hallmark study on central sensitization, Clifford Woolf stated: “an important question that still needs to be determined is whether there are individuals with a higher inherited propensity for developing central sensitization than others, and if so, whether this convey an increased risk in both developing conditions with pain hypersensitivity, and their chronification.”[47] This is clearly an important research issue. The present study may shed light on the role of the sensitization phenotype and enable prediction of the response to naloxone-induced reinstatement with the use of an enriched design. This design distributes participants as high sensitizers or low sensitizers (subjects presenting with large or small SHA, respectively, after a thermal injury). We anticipate that high sensitizers will more consistently develop reinstatement of hypersensitivity as compared with low sensitizers. If so, then this enriched design may be used in future clinical and basic science research to elucidate markers of vulnerability to chronic postsurgical pain.[16,17]

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