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CHAPTER V

No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery


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ABSTRACT

Serotonin–norepinephrine reuptake inhibitors inhibit the reuptake of serotonin and noradrenalin and are used in the treatment of neuropathic pain. Animal studies suggest that milnacipran co-administered with opioids may potentiate the analgesic effect of μ-opioid receptor agonists. This study hypothesised that co-administration of milnacipran and buprenorphine would have a synergistic effect in evoked pain models in healthy subjects. This was a randomised double-blinded, placebo-controlled, four-way cross-over, multiple dose clinical trial to investigate the analgesic effects of buprenorphine (placebo, 0.5, 1 and 3 μg kg⁻¹) in combination with milnacipran (placebo, 25 and 50 mg) in healthy subjects. 11 healthy men were enrolled in the study. Buprenorphine alone showed a dose-response relationship indicative of anti-nociception in the pain tests. Following milnacipran administration no changes were seen in the pharmacodynamic measurements for pain, psychomotor function, body stability or eye movements. For the electrical tests, cold pressor test and pressure pain test, buprenorphine alone was superior when compared with buprenorphine plus milnacipran. No differences in pharmacodynamic variables, besides an increase in pupil/iris ratio, were observed after repeated administration of milnacipran 50 mg. Single and multiple doses of 25 or 50 mg milnacipran did not further potentiate the anti-nociceptive effects of buprenorphine. Buprenorphine showed dose-dependent effects consistent with its pharmacological profile. Milnacipran alone did not affect any of the pain variables. The combination of both buprenorphine and milnacipran did not potentiate or show a synergistic effect on the pain models used in this study.

INTRODUCTION

Severe pain represents an important challenge for the clinician. Guidelines for moderate to severe pain treatment recommend the use of opioids. However, these can lead to dose-dependent side effects such as constipation, nausea, vomiting and sedation.¹

Current strategies in opioid use in the clinic include administering the lowest dose possible with still an adequate analgesic effect. An alternative strategy is to combine opioids with other drugs that might have a synergistic effect, which could thus lead to lower opioid dosages and therefore fewer side effects. Some suggested combinations are opioids in combination with norepinephrine transporter modulators, calcium channel alpha-2 delta ligands or local anesthetics.²

It has been demonstrated that milnacipran, a serotonin-noradrenaline reuptake inhibitor (SNRI), inhibits C-fibre-mediated nociceptive synaptic transmission in the spinal dorsal horn after the establishment of spinal long term potentiation in a neuropathic pain model, by activating both spinal serotonergic and noradrenergic systems.³ The inhibition of the C-fibre-mediated transmission by milnacipran could provide new evidence regarding the analgesic mechanism of SNRIs in chronic pain. Currently, milnacipran is available as a pharmacological intervention to treat chronic neuropathic pain and fibromyalgia; however its effectiveness in the treatment of pain is limited.³,⁵

There is nonclinical evidence that milnacipran potentiates the antihyperalgesic effects of opioids such as tramadol.⁵ Furthermore, in animal studies the antihyperalgesic effects of milnacipran can be blocked by naloxone, an opioid receptor antagonist, suggesting a possible opioidergic mechanism of action of this SNRI.⁷ This is supported by findings that show that noradrenergic, serotonergic and endogenous opioidergic systems are essential for milnacipran to reduce mechanical hyperalgesia.⁶

Serotonin-norepinephrine reuptake inhibitors (SNRIs) have shown effectiveness in human evoked pain models (single electrical stimulation and repetitive electrical stimulation) with venlafaxine, even with a short period of time (1 h) between the administration and the measured effect.⁸ Numerous human evoked pain models are sensitive to the effects of opioids⁹. Currently tapentadol, a drug that is both a μ-opioid receptor agonist and a noradrenaline reuptake inhibitor, is marketed for the treatment of severe acute and chronic neuropathic pain. Synergy between the dual mechanism of action of tapentadol has been demonstrated in several preclinical studies.¹⁰
This study aimed to evaluate the potential synergy and potentiation – as shown in preclinical studies – of milnacipran, when co-administered with a potent μ-opioid receptor partial agonist, buprenorphine, in evoked pain models in healthy subjects. This study aimed to investigate whether a sub-therapeutic dose of buprenorphine could become therapeutic through co-medication with single or multiple doses of milnacipran and to determine whether the analgesic effect of buprenorphine at therapeutic dose levels could be enhanced by milnacipran.

METHODS

The study was approved by the Medical Ethics Committee of the Bebo Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (Wmo) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. The trial was registered in the European Union Clinical Trials Register (2012-002302-43).

Subjects

Healthy male subjects between 18 and 45 years with a body mass index of 18-30 kg m⁻² were to be enrolled after having given written informed consent. The subjects underwent a full medical screening, including medical history taking, a physical examination, blood chemistry and haematology, urinalysis and electrocardiogram (ECG) to assess eligibility. Key exclusion criteria were as follows: clinical significant abnormalities during screening, regular user of any illicit drugs or history of drug abuse, a positive drug screen at screening or smoking within 3 months prior to screening. Use of xanthine-containing products and alcohol was not allowed during the stay at the research unit. Subjects were not allowed to use any medication from one week prior to the start of the study days.

Study design and treatments

This was a randomised, double-blind, placebo-controlled, four-way cross-over study with three different doses of buprenorphine or placebo in combination with milnacipran or placebo. The total number of planned subjects was 10.

The four treatment arms were as follows: buprenorphine active treatment in combination with milnacipran 25 mg (BUP+MIL-25), buprenorphine active treatment in combination with milnacipran 50 mg (BUP+MIL-50), buprenorphine-placebo in combination with milnacipran 50 mg (BUP+MIL-50) and buprenorphine active treatment in combination with milnacipran-placebo (BUP+MIL-P). The computer-generated randomization list was prepared by the statistician prior to the start of the study. Doses were prepared by a pharmacist/technician not involved in any of the study procedures. Buprenorphine (Temgesic; RB Pharmaceuticals Limited, Slough Berkshire, UK) was administered as an intravenous solution on day 1 and day 8 in three different doses. The buprenorphine dosing schedule was based on a published population pharmacokinetic and pharmacodynamic (PPPK/PD) model with the electric pain tolerance threshold as a pharmacodynamic endpoint. At the end of each buprenorphine intravenous infusion, the pharmacodynamic effects of buprenorphine were expected to remain reasonably stable, which would allow the performance of the pain tests (Figure 1). First, a 30 min 0.5 µg kg⁻¹ infusion, which was expected to lead to subtherapeutic plasma concentrations, followed 1.5 h later by a second 30 min 1 µg kg⁻¹ infusion, which was expected to lead to minimally therapeutic plasma concentrations, finally followed 1.5 h later by a 30 min 3 µg kg⁻¹ infusion, which was expected to lead to therapeutic plasma concentrations of buprenorphine. Milnacipran hydrochloride (Pierre Fabre, Castres, France) was administered orally twice daily starting from day 1 and until the morning of day 8. Intravenous metoclopramide 10 mg (Primperan; Sanofi-Aventis, Paris, France) was administered prophylactically before the second buprenorphine/placebo infusion to prevent nausea and vomiting. Additional doses of metoclopramide were administered if needed.

Each of the four study periods lasted 8 days. On the morning of day 1, subjects arrived at the clinical research unit and received the first oral dose of milnacipran or placebo. Thereafter, they received the three intravenous administrations of buprenorphine or placebo according to the different infusion schedules, separated by 1.5 h. After each infusion, the pharmacodynamic tasks were performed (evoked pain tasks and neurophysiological tests). At the end of the study day, subjects were discharged and were instructed to orally administer milnacipran or placebo twice a day at home. On day 8, subjects returned to the unit and the same procedures as on day 1 were followed. There was a 14-day wash-out interval between study periods. An overview of a study period is shown in Figure 2.
Pharmacodynamics

Pharmacodynamic measurements were performed pre-dose (twice) and 1 h after the start of each buprenorphine administration. A training session was included as part of the screening examination to reduce learning effects during the study. All measurements were performed in a quiet room with ambient illumination. Tests were performed in a fixed order (Figure 3).

Noceptive (pain) detection and tolerance thresholds were measured using a battery of evoked pain tasks. The battery is an integrated range of tasks for measuring different modalities of noception (electrical pain, pressure pain and cold pressor tasks). It aims to assess as objectively as possible the levels of pain induced by several noxious mechanisms in human subjects. All noceptive tests had previously been shown to be sensitive to the effects of analgesics in healthy adults. Other pharmacodynamic tests included pupil size measurements, adaptive tracking, saccadic eye movements and body sway. These tests have previously been shown to be sensitive to the effects of several different classes of drugs.

Pain intensity was measured continuously (beginning from when the first stimulus was applied until the predetermined end of the test) for each noceptive task using an electronic visual analogue scale (eVAS) ranging from 0 (no pain) to 100 (most intense pain tolerable). Equipment was programmed to cease giving stimuli if pain intensity reached the maximum possible score. For each task the pain detection threshold (PDT), pain tolerance threshold (PTT) and area under the pain intensity-stimulation (-time for cold pressor) curve (AUC) were calculated.

Electrical stimulation task

For cutaneous electrical pain, Ag-AgCl electrodes (3M Red-Dot™) were placed on cleaned, scrubbed, and if required, shaved skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be <2 kΩ. The electrical stimulus was delivered as two different paradigms by a computer-controlled constant current stimulator (DS5; Digitimer, Cambridge, UK).

For the single stimulus, adapted from methods previously described,14,15 (10 Hz tetanic pulse with a duration of 0.2 ms), current intensity increased from 0 mA in steps of 0.5 mA s⁻¹ until the pain tolerance threshold was reached or up to a cutoff of 50 mA.

For the repeated stimulus, adapted from methods previously described,16 each single stimulus (train of five, 1 ms square wave pulses repeated at 200 Hz) was repeated five times with a frequency of 2 Hz at the same current intensity with a random interval of 3 to 8 seconds between the repetitions. Current intensity increased from 0 to 50 mA in steps of 0.5 mA. Pain detection threshold (PDT) was taken as the value (mA) whereby a subject indicated either: all five stimuli were painful, or the train of five stimuli started feeling non-painful but ended feeling painful (VAS > 0). The pain intensity for each stimulation was measured using the eVAS slider, until pain tolerance threshold was reached or a maximum of 50 mA was reached.

Pressure stimulation task

The method of mechanical pressure pain induction was based on methods previously described and was shown to primarily assess noception generated from the muscle with minimal contribution by cutaneous nociceptors.17,18 Briefly, an 11 cm wide tourniquet cuff (VBM Medizintechnik GmbH, Sulz, Germany) was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa s⁻¹. The pneumatic pressure was increased until the subject indicated maximum pain tolerance using the eVAS slider, or a maximum pressure of 100 kPa was achieved, at which point the device released pressure to the cuff.

Cold pressor task

The method of cold pressor pain was based on the methods previously described19,20 and is the most commonly used test to induce inhibitory conditioned pain modulation (ICPM, also known as diffuse noxious inhibitory control).21 Subjects placed their non-dominant hand into a water bath (minimal depth 200 mm) (Lauda, Germany) at 35 ± 0.5°C for 2 min. At 1 min 45 s, a blood pressure cuff on the upper-arm was inflated to 20 mmHg below resting diastolic pressure. At 2 min, the subject then moved that hand from the warm water bath, directly into a similar sized bath at 1.0 ± 0.5°C. The subjects were instructed to indicate when PDT was reached (first change in sensation from cold non-painful to painful) as well as the pain intensity, by moving the eVAS slider. When PTT or a time limit (120 s) was reached, subjects were instructed to remove their hand from the water, at which point the blood pressure cuff deflated.
Conditioned pain modulation

Conditioned pain modulation is the activation of the pain-modulatory mechanism, as part of the descending endogenous analgesia system. The degree of ICPM was assessed by comparing the electrical pain thresholds for the single stimulus paradigm before and within 5 min after the end of cold pressor task.

Pupil size

Pupil diameter was determined using a digital camera and a flash. The pupil/iris ratio was calculated as a measure of pupil size (Qpupil, Leiden University Medical Center, Leiden, the Netherlands).

Adaptive tracking

The adaptive tracker is a psychomotor task and is sensitive to impairment of eye-hand coordination. The adaptive tracking test was performed as originally described by Borland and Nicholson, using customised equipment (Hobbs & Strutt, UK). A circle moves randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance over 3.5 min was used for analysis. The outcome is the average velocity of the circle as percentage of maximal velocity possible.

Saccadic eye movements

Saccadic peak velocity is one of the most sensitive parameters for sedation and was described previously. Recording and analysis of saccadic eye movements was conducted with a microcomputer-based system for sampling and analysis of eye movements. The program for signal collection and the A/D-converter was from Cambridge Electronic Design (CED Ltd., Cambridge, UK), the amplification by Grass (Grass-Telefactor, An Astro-Med, Inc. Product Group, Braintree, MA, USA) and the sampling and analysis scripts were developed at CHDR (Leiden, the Netherlands). Disposable silver-silver chloride electrodes (Ambu BlueSensor N, Ballerup, Denmark) were applied on the forehead and beside the lateral canthi of both eyes of the subject for registration of the electro-oculographic signals. The target consists of a moving dot that is displayed on a computer screen. Saccadic eye movements were recorded for stimulus amplitudes of approximately 15 degrees to either side. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 s. Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were analysed.

Body sway

The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with pot string meter (Celesco, Chatsworth, CA, USA) based on the Wright ataximeter. Subjects were asked to stand still and comfortable, with their feet approximately 10 cm apart and their hands in a relaxed position alongside the body and eyes closed. With a string attached to the waist, all body movements over a period of two min were integrated and expressed as mm sway.

Measurements of drug concentrations in plasma

Samples for determination of milnacipran in plasma were obtained at baseline, 1, 2, 3, 4, 5, 6, 7 and 11 h after oral administration on days 1 and 8. Samples for determination of buprenorphine and its active metabolite nor-buprenorphine were obtained 1 and 2 h after the start of each infusion. Samples were collected in lithium heparin tubes and stored in ice. Plasma was separated within 30 min of blood collection by centrifugation at 2000 g for 10 min. Samples were stored at -70°C until analysis. Drug concentrations in plasma were determined using a validated Liquid Chromatography–Mass Spectrometry (LC-MS/MS) technique. The analytical range of the assay was 1.00-500 ng/mL for milnacipran and 0.100-20.0 ng/mL for buprenorphine and nor-buprenorphine.

Statistics

The sample size calculation was based on previous experiments in healthy young men. An average cold pressor AUC of 10,000 s mm was expected. We expected that the highest dose of buprenorphine would cause a decrease in the cold pressor AUC of 30%. If an increase of that difference to 37% was to
Pharmacokinetic analysis was performed using a non-compartmental model approach. For milnacipran and buprenorphine, the peak concentration (Cₘₚ) and the time to the peak concentration (tₘₚ) was recorded as observed. In addition, the area under the plasma concentration-time curve from time zero to the time of the last sample (AUC₀₋ₗₚ) was determined for both drugs. Calculations were performed using R v2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

The pharmacodynamic data were compared, per day, with a mixed model analysis of variance with treatment, period, time and treatment by time as fixed factors, subject, subject by treatment and subject by time as random factors and the average pre-value (per treatment average of measurements before time=0 on day 1, also for the day 8 analysis) as covariate. This analysis was carried out on the four original treatments. The contrast between buprenorphine alone and buprenorphine and milnacipran 50 mg during the baseline measurements on day 8 was assessed to determine the effects of repeated milnacipran dosing.

Variables that followed a log-normal distribution were log-transformed before analysis. Transformed parameters were back-transformed after analysis.

Synergy was tested as the contrast of (buprenorphine alone) plus (milnacipran 50 mg alone) minus the overall average pre value (day 1) versus (buprenorphine and milnacipran 50 mg). The average overall pre-value of day 1 is used as there is no milnacipran-placebo and buprenorphine-placebo treatment. The values of the buprenorphine alone plus milnacipran 50 mg alone minus the overall average pre-value were calculated prior to analysis. Together with the buprenorphine plus 25 mg milnacipran and the buprenorphine plus 50 mg milnacipran, the calculated synergy values were analysed in a separate repeated measure mixed model, with fixed factors of treatment, time and treatment by time, random factors of subject, subject by treatment and subject by time and the average pre-value per treatment of day 1 as covariates. All calculations of the pharmacodynamic parameters were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The main SAS procedure that was used in the analysis was PROC MIXED. No adjustments for multiple comparisons were employed.

RESULTS

A total of 11 subjects participated in the trial (see Figure 51 for study flow-chart); subjects were aged 21-31 years (mean age 24.2 years) and had a body mass index of between 20 and 26 kg m⁻² (mean 22.4 kg m⁻²). Nine subjects completed the trial until the last study occasions. Two subjects dropped out from the study. One subject was withdrawn by the investigator due to side effects categorised as probably related to milnacipran (shortness of breath, palpitations, dizziness, urinary hesitation and paraesthesias). The other subject withdrew consent due to side effects (nausea and vomiting) caused by buprenorphine.

Nociceptive tests

The least squares means and the analysis results for the PTTS for the different nociceptive tests are presented in Table 1, Table 2 and figure 4. On day 1, a significant overall treatment effect was found for the PTTS of the electrical stimulation tasks, the pressure stimulation task and the cold pressor tasks. On day 8, no significant overall treatment effect was found for any of the pain tests. None of the contrasts of buprenorphine plus milnacipran versus buprenorphine alone showed a significant increase of pain tolerance or detection. Buprenorphine in combination with milnacipran 50 mg significantly decreased the repeated electrical stimulation PTTS and the cold pressor PTTS compared with buprenorphine alone (PTTS data not shown). The effects were not observed on day 8. Buprenorphine in combination with milnacipran did not lead to greater analgesic effects compared with buprenorphine alone. On day 8, buprenorphine in combination with milnacipran 50 mg and buprenorphine in combination with 25 mg milnacipran increased the cold pressor PTTS compared with buprenorphine alone. The contrast between buprenorphine in combination with milnacipran 50 mg and 25 mg after 8 days of repeated dosing was statistically significant; however no overall treatment effect was observed for the cold pressor test on day 8 (p = 0.077).

No treatment effects could be observed on iCPM on day 1 or day 8. Repeated milnacipran dosing did not affect any of the pain variables during the day 8 baseline measurements.

Synergy between treatments was assessed in a separate analysis (Table 3). No synergy was observed when buprenorphine and milnacipran were administered together, either after a single dose or after repeated dosing.
Pharmacodynamic tests of psychomotor function, body stability, eye movements and pupil size

The least squares means and the analysis results for pharmacodynamic tests for psychomotor function, body stability, eye movements and pupil size are presented in Table 4 and Figure 5. The pupil/iris ratio decreased in all treatment groups receiving buprenorphine both on day 1 and day 8, compared with milnacipran alone. On day 1, no differences were observed in pupil size between buprenorphine/milnacipran combination groups compared with buprenorphine alone. On day 8, the pupil/iris ratio was significantly larger in the buprenorphine/milnacipran combination groups compared with buprenorphine alone after the first and second buprenorphine dose. Saccadic peak velocity and adaptive tracking performance decreased after receiving the buprenorphine combinations compared with milnacipran alone. Milnacipran did not significantly potentiate the decrease due to buprenorphine in saccadic peak velocity or adaptive tracking. A significant treatment effect was observed for the body sway measurements on day 8, and no significant differences between buprenorphine combinations versus buprenorphine alone were observed. Repeated milnacipran dosing only lead to an increase in pupil/iris ratio (0.071; 95% CI 0.024-0.119; p = 0.0045; data not shown). No differences in eye movement, psychomotor function or body stability were observed during day 8 baseline measurements.

Pharmacokinetics

The sampling of buprenorphine and milnacipran was intended to corroborate adequate exposure and to detect possible pharmacokinetic interactions when co-administered. Samples taken after the first buprenorphine infusion were not analysed as it was expected that these samples would be below the lower limit of quantification. After the second and third buprenorphine infusions the measured concentrations were as expected. The mean (population) expected buprenorphine concentrations values of the pharmacokinetic model that was used to define the study is shown with the actual concentrations achieved in the study also plotted on top (Figure 6). The samples for buprenorphine near to the trough show a slight overestimation in comparison to the prediction by the model. Samples were also assayed for nor-buprenorphine but due to its low concentration in plasma, all determinations were below the lower limit of quantification (LLOQ).

Pharmacokinetic parameters for milnacipran on days 1 and 8 after administration are shown in Table 5. C_{max} and AUC_{0-\text{last}} for milnacipran increased in a dose dependent manner. AUC_{0-\text{last}} and C_{max} were approximately two-fold greater on day 8 compared with day 1.

Safety

As shown in Table 6, all subjects experienced at least one treatment emergent adverse event in each study period. The most reported adverse events were nausea, somnolence and vomiting. Vomiting occurred in all subjects receiving buprenorphine and the high dose of milnacipran. In subjects only receiving milnacipran 50 mg, only three subjects reported vomiting.

DISCUSSION

The study aimed to evaluate the possibility of potentiation or synergy of milnacipran, when co-administered with buprenorphine in evoked pain models in healthy subjects, both after single and multiple doses of milnacipran. Furthermore, the interaction of both compounds on psychomotor function, body stability, eye movements and pharmacokinetic parameters and safety was investigated.

Nociceptive tests and measurements of psychomotor function, body stability and eye movements following buprenorphine administration were consistent with the drug being a partial opioid receptor agonist. Buprenorphine showed a dose-response relationship indicative of antinociception for the variables evaluated in the electrical, cold and pressure tests. Also, a decline in the performance was seen in the adaptive tracker and saccadic eye movements after the buprenorphine administration, indicative of a mild level of sedation. Following single and repeated milnacipran (alone) administration, no changes were observed in the nociceptive tasks or other pharmacodynamic measurements. No earlier studies were performed with milnacipran using pain models. Effects of other SNRIs in human pain models have been reported before. Venlafaxine increased pain tolerance thresholds on single electrical stimulation and pain summation on repetitive stimulation; however in that study no differences on the cold pressor test and on a pressure pain paradigm could be observed. These reported findings could not be replicated in our study.

Acute, single doses of 25 and 50 mg milnacipran did not potentiate the antinociceptive effects or the effects on psychomotor function, body...
A significant difference in the effect on cold pressor ptt was observed nacipran on the pain variables or the other pharmacodynamic variables. Potentiation or synergy was observed between buprenorphine and milnacipran 50 mg alone, which may be an indication of less sedation. In fact, for the repeated electrical stimulation task (repeated stimulus), the combination buprenorphine and milnacipran 50 mg lead to a lower ptt compared with buprenorphine alone. For the saccadic peak velocity, the addition of milnacipran 50 mg diminished the pharmacodynamic effects (i.e. decrease in saccadic peak velocity) observed after buprenorphine alone, which may be an indication of less sedation.

On day 8, after repeated administration of milnacipran 25 or 50 mg, no potentiation or synergy was observed between buprenorphine and milnacipran on the pain variables or the other pharmacodynamic variables. A significant difference in the effect on cold pressor ptt was observed between buprenorphine alone and buprenorphine in combination with milnacipran 25 and 50 mg on day 8. However, no overall significant treatment effect was observed for the cold pressor ptt on day 8. Moreover, no adjustments for multiple testing were applied in this study, so we consider this finding not sufficiently indicative of the existence of potentiation due to the combined treatment with buprenorphine and milnacipran.

The pupil/iris ratio decreased after treatment with buprenorphine (with and without co-administration of milnacipran). However, after 8 days of treatment with milnacipran 50 mg, the pupil/iris ratio after the first and second buprenorphine infusion was significantly larger in the buprenorphine/milnacipran combination group compared with buprenorphine alone. Miosis after treatment with buprenorphine is a well-known effect of μ-opioid receptor agonists.

Mydriasis after treatment with an SNRI has been shown before for duloxetine and for venlafaxine. Here, we show both the mydriasis effect of milnacipran after 8 days of treatment and the acute miosis effect of buprenorphine. The mydriasis effect of milnacipran could only be reversed at the highest dose of buprenorphine.

In the cases that an overall treatment effect was observed for nociceptive endpoints on day 1, which were due to the effect of buprenorphine versus no buprenorphine, no overall treatment effects were observed on day 8. This may indicate that some tolerance may have occurred for the antinociceptive effects of buprenorphine or an increased (but not significant) effect of milnacipran after 8 days of treatment. In contrast, for the endpoints of psychomotor function, body stability and eye movements, when an overall treatment (buprenorphine) effect was observed on day 1, it was also observed on day 8.

Milnacipran has a modulating effect on serotonin (5-HT) and norepinephrine (NE) neurotransmitters. 5-HT and NE are involved in the modulation of endogenous analgesic mechanisms via inhibitory pain pathways in the central nervous system. Opioids can also influence descending pain pathways. Earlier, it was shown that buprenorphine is able to potentiate iCpm in a human pain model. This study was not able to replicate these findings. No effects were observed in conditioned pain modulation after treatment with milnacipran, buprenorphine or the combination of the two.

In animal studies, milnacipran inhibited C-fibre-mediated nociceptive synaptic transmission in the spinal dorsal horn after the establishment of spinal long term potentiation in a neuropathic pain model. Furthermore, it has been shown that milnacipran reduces thermal and mechanical allodynia in a rat model of neuropathic pain (chronic constriction injury of the sciatic nerve). However, in another model of neuropathic pain in mice (central post-stroke pain model), milnacipran did not affect mechanical allodynia thresholds. Co-administration of milnacipran with tramadol potentiated the antihyperalgesic effect of tramadol, which used chronic constriction injury model as a model for neuropathic pain. Considering the effects of milnacipran in several hyperalgesia models in animals, a pain model which is able to measure antihyperalgesia in humans would have been a valuable addition to this study.

Only models for acute pain were used in this study. It is important to note that differences exist between these models and clinical chronic (neuropathic) pain. In a recent review Lötsc et al. suggested that hyperalgesia and electrical pain models might be used to predict clinical analgesia in neuropathic pain. However, several pharmacological (NMDA receptor antagonists, tricyclic antidepressants, SNRIs, gabapentin) and non-pharmacological therapies (such as repetitive transcranial stimulation) that are used to treat neuropathic pain have shown contradictory results on acute pain models. Therefore, although we were not able to show potentiation or synergy in these acute pain models, no conclusions can be drawn on a possible synergistic effect of milnacipran and buprenorphine in chronic pain conditions.

In the milnacipran pharmacokinetic analysis, Cmax and AUC0-last for milnacipran increased in a dose proportional manner. Milnacipran showed accumulation after 7 days of treatment with 25 or 50 mg twice a day and resulted in higher concentrations in all subjects on day 8 of treatment. For buprenorphine, no formal pharmacokinetic analysis was possible due to
the sparse sampling. The plasma buprenorphine concentrations were in agreement with what was expected based on the simulation prior to the study. No pharmacokinetic interaction was observed between milnacipran and buprenorphine.

This was a four-way crossover study and no study period with a double placebo was included. To assess synergy between buprenorphine and milnacipran, the milnacipran only plus the buprenorphine only value minus the average pre-value over all occasions were calculated. The buprenorphine plus 25 mg milnacipran, the buprenorphine plus 50 mg milnacipran and the new values of milnacipran plus buprenorphine minus pre-values were analysed in a separate repeated measure mixed model. No synergy between these treatments could be observed, although it can be argued that the lack of a complete placebo profile made formal testing for synergy difficult.

In conclusion, buprenorphine showed dose-dependent effects consistent with its pharmacological profile; antinociception and a decrease in neurophysiological functions. Milnacipran alone did not affect any of the pain variables. The combination of both buprenorphine and milnacipran did not potentiate or show a synergistic effect on the pain models used in this study. No conclusions can be drawn on a possible synergistic effect of milnacipran and buprenorphine in clinical, chronic pain conditions.

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### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LS Means (day 1)</th>
<th>LS Means (day 8)</th>
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<td>Electrical Repeat PTT (mA)</td>
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<td>Electrical Single PTT (mA)</td>
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<td>Pressure PTT (kPa)</td>
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<td>Cold Pressor PTT (s)</td>
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<td>Left Pupil/Iris ratio</td>
<td>0.328</td>
<td>0.304</td>
</tr>
<tr>
<td>Saccadic Peak Velocity (deg s⁻¹)</td>
<td>428.1</td>
<td>420.3</td>
</tr>
<tr>
<td>Body Sway (mm)</td>
<td>387.5</td>
<td>399.3</td>
</tr>
</tbody>
</table>
### Table 2

Estimates of difference, 95\% confidence intervals and \(p\)-values for main contrasts for nociceptive measurements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Contrast (day 1)</th>
<th>Contrast (day 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>BUP+MIL-50 vs BUP+MIL-25</td>
<td>BUP+MIL-50 vs BUP+MIL-25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BUP+MIL-50 vs BUP+MIL-50</td>
<td>BUP+MIL-50 vs BUP+MIL-50</td>
</tr>
</tbody>
</table>

- **Electrical Repeated PTT (mA)**
  - Overall: 0.0066, 0.05 (94.8\% CI: 44.8\% – 84.8\%)
  - After second: 0.0381 (94.8\% CI: 44.8\% – 84.8\%)
  - After third: 0.0362 (94.8\% CI: 44.8\% – 84.8\%)

- **Pressure PTT (kPa)**
  - Overall: 0.0195, 0.21 (97.2\% CI: 94.6\% – 99.7\%)
  - After second: 0.0195, 0.21 (97.2\% CI: 94.6\% – 99.7\%)
  - After third: 0.0195, 0.21 (97.2\% CI: 94.6\% – 99.7\%)

### Table 3

Estimates of difference, 95\% confidence intervals and \(p\)-values for contrasts to assess synergy for the nociceptive measurements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ls Means (day 1)</th>
<th>Contrasts (day 1)</th>
<th>ls Means (day 8)</th>
<th>Contrasts (day 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>BUP+mil-50</td>
<td>PRE</td>
<td>BUP+mil-50</td>
</tr>
<tr>
<td></td>
<td>BUP+mil-25</td>
<td>(BUP+MIL-50)</td>
<td>(BUP+MIL-50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BUP+mil-50</td>
<td>(BUP+MIL-50)</td>
<td>(BUP+MIL-50)</td>
<td></td>
</tr>
</tbody>
</table>

- **Electrical Repeated PTT (mA)**
  - Overall: 12.68, 11.37
  - After second: 12.68, 11.37

- **Pressure PTT (kPa)**
  - Overall: 52.83, 51.95
  - After second: 52.83, 51.95

- **Conditioned Pain Modulation (mA)**
  - Overall: 152, 152
  - After third: 152, 152
the use of a battery of evoked pain models in early phase drug development

Buprenorphine and milnacipran interaction study in healthy subjects

Table 4
Estimates of difference, 95% confidence intervals and p-values for main contrasts for the neuropsychological measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>p-value</th>
<th>contrast (day 1)</th>
<th>contrast (day 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupil/Iris ratio (left)</td>
<td>Overall</td>
<td>&lt;.0001</td>
<td>-0.027</td>
<td>-0.070</td>
</tr>
<tr>
<td></td>
<td>After first</td>
<td>0.020</td>
<td>p=0.3011</td>
<td>-0.015</td>
</tr>
<tr>
<td></td>
<td>After second</td>
<td>0.011</td>
<td>p=0.0081</td>
<td>-0.065</td>
</tr>
<tr>
<td></td>
<td>After third</td>
<td>0.013</td>
<td>p=0.0081</td>
<td>-0.011</td>
</tr>
<tr>
<td>Saccadic Peak Velocity</td>
<td>Overall</td>
<td>0.0005</td>
<td>-27.0 (-59.5, 5.4)</td>
<td>-2.2 (-21.1, 17.9)</td>
</tr>
<tr>
<td></td>
<td>After first</td>
<td>-22.0</td>
<td>p=0.0004</td>
<td>-23.0 (-21.1, -24.9)</td>
</tr>
<tr>
<td></td>
<td>After second</td>
<td>-35.6</td>
<td>p=0.0004</td>
<td>-24.1 -24.7</td>
</tr>
<tr>
<td></td>
<td>After third</td>
<td>-45.4</td>
<td>p=0.0004</td>
<td>-1.8 -12.1</td>
</tr>
<tr>
<td>Body Sway (mm)</td>
<td>Overall</td>
<td>0.3182</td>
<td>-14.2% 10.8% 0.0338</td>
<td>12.3% -2.4%</td>
</tr>
<tr>
<td></td>
<td>After first</td>
<td>0.4018</td>
<td>p=0.0004</td>
<td>-9.5% -9.5%</td>
</tr>
<tr>
<td></td>
<td>After second</td>
<td>0.2873</td>
<td>p=0.0004</td>
<td>24.1 24.1</td>
</tr>
<tr>
<td></td>
<td>After third</td>
<td>0.0131</td>
<td>p=0.0004</td>
<td>6.3% 6.3%</td>
</tr>
<tr>
<td>Adaptive Tracking (%)</td>
<td>Overall</td>
<td>&lt;.0001</td>
<td>-3.46 0.46 5.45</td>
<td>-3.46 0.46 5.45</td>
</tr>
<tr>
<td></td>
<td>After first</td>
<td>0.051</td>
<td>p=0.0004</td>
<td>-2.39 -2.39</td>
</tr>
<tr>
<td></td>
<td>After second</td>
<td>-0.34</td>
<td>p=0.0004</td>
<td>-0.34 -0.34</td>
</tr>
<tr>
<td></td>
<td>After third</td>
<td>-3.60</td>
<td>p=0.0004</td>
<td>-1.22 -1.22</td>
</tr>
</tbody>
</table>

Table 5
Plasma pharmacokinetic parameters for milnacipran

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>46.9 ± 15.3</td>
<td>99.8 ± 37.7</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>3.2 ± 0.8</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>AUC(last) (ng hr/ml)</td>
<td>468.3 ± 202.3</td>
<td>663.0 ± 314.4</td>
</tr>
</tbody>
</table>

Table 6
Summary of treatment emergent adverse events (AEs) by frequency [n(%)]. AEs occurring more than 3 times within one treatment are reported.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>9 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>11 (100%)</td>
<td>9 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>8 (88.9%)</td>
<td>8 (88.9%)</td>
<td>8 (88.9%)</td>
<td>7 (63.6%)</td>
<td>9 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>8 (88.9%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
<td>7 (63.6%)</td>
<td>9 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 (66.7%)</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>6 (54.5%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
<td>9 (90.0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (44.4%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
<td>8 (80.0%)</td>
</tr>
<tr>
<td>Feeling hot</td>
<td>2 (22.2%)</td>
<td>3 (33.3%)</td>
<td>3 (33.3%)</td>
<td>3 (33.3%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Hot flash</td>
<td>3 (33.3%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>2 (22.2%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>2 (22.2%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>2 (20.0%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
</tr>
</tbody>
</table>
Simulated plasma buprenorphine concentrations and electrical pain tolerance threshold (12h). Black solid line represents the mean (population) plasma concentration after three different 0.5-h buprenorphine infusions in a 70-kg subject: 0.5 (subtherapeutic), 1 (minimum therapeutic) and 3 (therapeutic) μg kg⁻¹. Dotted line represents the pain tolerance threshold.

**Figure 1**

Study period overview.

**Figure 2**

Overview and sequence of pharmacodynamic tests.

- **Pressure Stimulation Task**
  - Tourniquet calf: 0.5 kPa s⁻¹, max 100 kPa

- **Electrical Stimulation**
  - Single stimulus 0.2 ms at 10 Hz
  - Repeated stimuli: train of 5 stimuli at 2 Hz
  - Both increase 0.5 mA s⁻¹, max 50 mA

- **Conditioned Pain Modulation**

- **Cold Pressor**
  - Forearm: 120 s in 35°C then 1°C in water

- **Electrical**
  - Single stimulus 0.2 ms at 10 Hz

- **Adaptive Tracking**

- **Saccadic Eye Movements**

- **Pupil Size**

- **Body Sway**

KPa, kilopascal; ms, millisecond; Hz, hertz; mA, milliampere; °C, degree Celsius.
FIGURE 4
Time course of the mean change from baseline profile in least squares means for the pain tolerance threshold for electrical stimulation (repeated stimulus) [A/B], pressure pain [C/D] and the cold pressor task [E/F] after administration of milnacipran (MIL), buprenorphine 0.5 µg kg⁻¹ (BUP 1), buprenorphine 1.5 µg kg⁻¹ (BUP 2) and buprenorphine 3.0 µg kg⁻¹ (BUP 3) on day 1 (left) and day 8 (right).

A
B
C
D
E
F

FIGURE 5
Time course of the mean change from baseline profile in least squares means for the left pupil/iris ratio [A/B] and saccadic peak velocity [C/D] after administration of milnacipran (MIL), buprenorphine 0.5 µg kg⁻¹ (BUP 1), buprenorphine 1.5 µg kg⁻¹ (BUP 2) and buprenorphine 3.0 µg kg⁻¹ (BUP 3) on day 1 (left) and day 8 (right).
FIGURE 6
Plasma buprenorphine concentrations and popPK buprenorphine model on day 8 of the trial. The circles represent the measured concentrations and the solid line represents the mean (population) buprenorphine concentration after three different 0.5-h buprenorphine infusions in a 70-kg subject: 0.5 (sub-therapeutic), 1 (minimum therapeutic) and 3 (therapeutic) μg kg⁻¹. Vertical lines at time points 0, 120 and 240 min represent the start of the infusion for buprenorphine. The horizontal discontinuous line represents the lower limit of quantification for buprenorphine.

FIGURE SUPPLEMENTARY 1
CONSORT Flowchart

- **ENROLLMENT**
  - Assessed for eligibility (n=24)
    - Excluded (n=7)
      - Screen failure (n=7)
      - Achieved tolerance >80% for maximum input of nociceptive test at training (n=2)
      - Blood pressure too high (n=3)
      - Lab abnormalities (n=1)
      - Smoker (n=1)
      - Withdrew consent during screening period (n=1)
      - Reserve subjects (n=5)

- **RANDOMISED** (n=21)

- **ALLOCATION**
  - Subjects enrolled to treatment (n=21):
    - BUP+MIL-25 (n=9)
    - BUP+MIL-50 (n=11)
    - BUP+MIL-P (n=10)
    - BUP-P+MIL-50 (n=11)

- **FOLLOW UP**
  - Subject completing period
    - BUP+MIL-25 (n=9)
    - BUP+MIL-50 (n=10)
    - BUP+MIL-P (n=9)
    - BUP-P+MIL-50 (n=11)
    - Completed all study periods (n=9)

- **ANALYSIS**
  - Analysed (n=11)
    - Excluded from analysis (n=0)
    - Dropped out from study (n=2)
      - Withdrawn by investigator (n=2), BUP-P+MIL-50
      - Subject withdrew consent due to side effects (n=1), BUP+MIL-P
  - Subjects enrolled to treatment (n=11):