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RESEARCH PAPER

Effect of transdermal opioids in experimentally induced superficial, deep and hyperalgesic pain

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BACKGROUND AND PURPOSE

Chronic pain and hyperalgesia can be difficult to treat with classical opioids acting predominately at the μ-opioid receptor. Buprenorphine and its active metabolite are believed to act through μ-, κ- and δ-receptors and may therefore possess different analgesic and anti-hyperalgesic effects compared with pure μ-receptor agonists, for example, fentanyl. Here, we have compared the analgesic and anti-hyperalgesic effects of buprenorphine and fentanyl.

EXPERIMENTAL APPROACH

Twenty-two healthy volunteers were randomized to treatment with transdermal buprenorphine (20 $\mu g \cdot h^{-1}$, 144 h), fentanyl (25 $\mu g \cdot h^{-1}$, 72 h) or placebo patches in a double-blind, cross-over experimental pain study. The experimental pain tests (phasic pain, sensitization) involved pressure at the tibial bone, cutaneous electrical and thermal stimulation, intramuscular nerve growth factor, UVB light burn injury model and intradermal capsaicin-induced hyperalgesia. Pain testing was carried out at baseline, 24, 48, 72 and 144 h after application of the drugs.

KEY RESULTS

Compared with placebo, buprenorphine, but not fentanyl, significantly attenuated pressure at the tibial bone as well as pressure pain in the primary hyperalgesic area induced by UVB light The two drugs were equipotent and better than placebo against cutaneous thermal pain stimulation), but failed to show significant analgesic effect to cutaneous electrical stimulation, nerve growth factor-induced muscle soreness and to capsaicin-induced hyperalgesia.

CONCLUSIONS AND IMPLICATIONS

Buprenorphine, but not fentanyl, showed analgesic effects against experimentally induced, bone-associated pain and primary hyperalgesia compared with placebo. These tissue- and modality-differentiated properties may reflect the variable effects of opioid drugs observed in individual patients.

Abbreviations

MED, minimal erythema dose; NGF, nerve growth factor; PDT, pressure detection threshold; PTT, pressure tolerance threshold; TDS, transdermal delivery system; UVB, ultra-violet B-light; VAS, visual analogue scale

Introduction

Studies in animals and humans indicate that μ-opioid receptor agonists such as morphine may paradoxically induce hyperalgesia and thereby exacerbate pain perception in patients (Celerier *et al*., 2000; Guignard *et al*. 2000; receptor nomenclature follows Alexander *et al*., 2009). Animal studies suggest that δ-opioid receptor agonists may have antihyperalgesic effects (Negus *et al*. 1989; Brainin-Mattos *et al*., 2006). Such studies have furthermore showed that δ-receptors play an important role in bone-associated nociception which in clinical practice is difficult to treat (Mizoguchi *et al*. 2003;

Delaney *et al*., 2008). New management approaches have therefore involved the use of opioids with a differential selectivity towards δ-receptors (Stein *et al*. 2003; De Schepper *et al*., 2004). Buprenorphine is an opioid with agonistic effect at the μ-receptors, variable effect at the κ-receptors and antagonistic effect at the δ-receptors, but with very low affinity to the δ-receptor (Johnson *et al*., 2005). Its active metabolite, norbuprenorphine, acts as a strong agonist at the δ-receptors (Huang *et al*., 2001; Kress 2009). Buprenorphine and norbuprenorphine may therefore be important in the treatment of hyperalgesia as well as bone-associated pain (Schutter *et al*., 2008).

Buprenorphine has been used in clinical practice for more than 30 years using various modes of administration such as intravenous, sublingual and spinal-epidural administration (Yassen *et al*., 2006). However, after the introduction of transdermal delivery system (TDS) of the drug, there has been a renaissance of interest in its analgesic profile. Through a constant rate of release of the drug, the TDS maintains stable plasma concentrations and it is therefore valuable in the treatment of patients with persistent pain. Buprenorphine and fentanyl are both potent opioids that are available as TDS preparations and are currently used in the treatment of chronic moderate to severe pain. There is some evidence that opioids have different analgesic and antihyperalgesic potencies and vary with respect to opioid-induced hyperalgesia (Koppert *et al*., 1999; 2005).

Due to the many confounding factors in patients, it can be difficult to evaluate the specific pain mechanisms of analgesics in patients with pain, and hence experimental standardized pain models need to be used (Arendt-Nielsen *et al*., 2007). Such models provide the possibility of investigating the tissue-differentiated effects of opioids (Staahl *et al*., 2009).

It was hypothesized that buprenorphine and fentanyl would modulate, differently, experimentally induced pain from superficial and deep tissues, and experimentally induced hyperalgesia. In our double-blind and placebo controlled cross-over study, the aims were: (i) to compare the analgesic and antihyperalgesic effect of TDS fentanyl and buprenorphine in various tissues over 72 h; and (ii) to investigate the effect of buprenorphine compared with placebo over 144 h.

Methods

The study was approved by the local Ethical Committee (N-20070061) and the Danish Medicines Agency (EudraCT number: 2007–004524-21). It was carried out according to the principles of Good Clinical Practice of the European Union. The study was of a double-blinded, randomized, placebo-controlled, three-arm, cross-over design.

Subjects and study design

Based on a similar study where transdermal buprenorphine and fentanyl revealed analgesic effect of the two drugs (Koltzenburg *et al*., 2006) 22 healthy opioid-naive male volunteers (mean age 23.1 ± 3.8 years) were included. The study was carried out in the research laboratories at Mech-Sense, Aalborg Hospital, Denmark. All subjects gave written informed consent before inclusion. None of them had any long-lasting pain complaints or lesions at the testing sites. Medical examinations and routine blood samples were normal.

Based on studies where morphine was used as comparator, buprenorphine and fentanyl were considered equipotent in the ratio 20 $\mu g \cdot h^{-1}$ buprenorphine: 25 $\mu g \cdot h^{-1}$ fentanyl (Sittl *et al*., 2005). This correlates to the clinical conversion rates and these concentrations were therefore used in the present study (Elsner *et al*., 1999; Likar *et al*. 2008). Each subject received 20 $\mu g \cdot h^{-1}$ buprenorphine (transdermal patch, Norspan® 144 h, Norpharma, Denmark), 25 $\mu g \cdot h^{-1}$ fentanyl (transdermal patch, Durogesic® 72 h, Hospital Pharmacy, Nord-Jutland, Denmark) or placebo (transdermal patch). In each period, two patches were applied. This was done to ensure adequate 'blinding' of the study as the buprenorphine and the fentanyl transdermal patches were not identical, that is, buprenorphine active or placebo patch was placed on the right shoulder and fentanyl active or placebo patch was placed on the left shoulder (Figure 1A). Treatment duration of buprenorphine was 144 h and for fentanyl it was 72 h. Therefore the patch placed on the right shoulder (buprenorphine active or placebo patch) was removed after 144 h and the patch placed on the left shoulder was removed after 72 h (fentanyl active or placebo patch). The patches were applied by either a nurse or a pharmacist, who were not otherwise involved in the project, and thus the investigator was not aware of the treatment given.

Each arm consisted of 10 days of treatment – 7 days of treatment and 3 days of follow-up. The experimental pain stimulations were performed during this treatment time (Table 1). Pain measurements were performed before application (baseline) and 24, 48, 72 and 144 h after application of the patches and were performed in the same order each time and in each treatment period (Table 1). There was 3–5 min lag time between the tests. However, as this could vary depending on a subject's well-being (adverse effects such as vomiting, nausea) it was not possible to standardize the lag time between the tests over the treatment duration of 144 h. All subjects went through all the tests. All subjects were hospitalized during the 7 day treatment period in case any adverse effects needed observation or treatment. Between each treatment arm there was a wash-out period of at least 10 days. During treatment blood pressure, heart rate, respiration and saturation were monitored daily. Adverse effects were registered daily throughout the period (see the section Adverse effects).

Before enrolment in the study the subjects met for a screening session, where a medical examination was completed, routine blood samples were taken and training of the pain stimulations was performed. Furthermore, the visual analogue scale (VAS defined as: 1 – vague perception or mild sensation; 2 – definite perception of mild sensation; 3 – vague perception of moderate sensation; 4 – definite perception of moderate sensation; 5 – pain detection threshold; 6 – slight pain; 7 – moderate pain or tolerance threshold; 8 – medium pain intensity; 9 – intense pain; and 10 – unbearable pain) was explained to the subjects, and they were trained in assessment of pain using the scale. After enrolment, pain measurements were performed before application (baseline) of the patches and 24, 48, 72 and 144 h after application

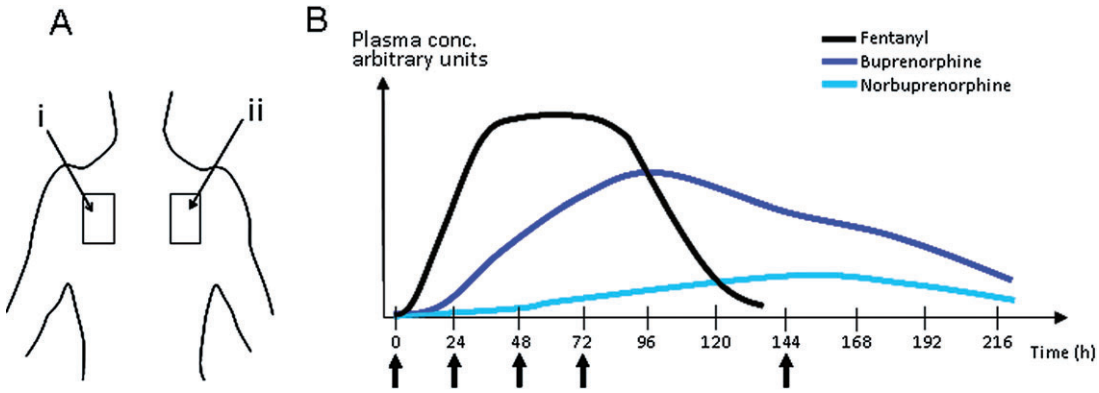

Figure 1

A: To ensure blinding of the study, two patches (one a placebo patch) were applied in each treatment period on the shoulders of the healthy volunteers. i) Either active fentanyl or placebo fentanyl patch. ii) Either active buprenorphine or placebo patch. B: The graph represents theoretical plasma concentrations of buprenorphine, its active metabolite norbuprenorphine and fentanyl. Arrows (0 = baseline, 24, 48, 72 and 144 h) represents the time at which pain measurements were performed in each of the three treatment periods.

Table 1

Pain measurements were performed before application (baseline) and 24, 48, 72 and 144 h after application of the drugs

Pain test	Baseline	24 h	48 h	72 h	144 h
Superficial/deep pain					
Tibia	x	x	x	x	x
Heat	x	x	x	x	x
Electrical	x	x	x	x	x
Hyperalgesia/Inflammation					
UVB	(x)	x	(x)	x	x
NGF	x	x	x	x	x
CAP	x			x	x

Order of testing: (i) pressure at the tibia; (ii) pressure at the NGF site; (iii) assessment of secondary hyperalgesic area to UVB-induced first-degree sunburn; (iv) pressure in the primary area of UVB; (v) heat stimulation in the primary area of UVB; (vi) heat stimulation on the arm; (vii) assessment of secondary hyperalgesic area and allodynic area to capsaicin injection in the arm; and (viii) electrical stimulation at the median nerve.
Tibia, pressure at the tibial bone; UVB, ultraviolet B-radiation; NGF, nerve growth faction (i.m. injection); CAP, capsaicin (i.d. injection); x, performing a pain measurement; (x), stimulating with UVB light prior to measurement of sensitivity (measurement was performed 24 h after).

(Figure 1B). The stimulations were performed in same order at each time. To ensure that the same stimulation site was used in each treatment period all stimulation sites were marked and drawn on a transparency film. Venous blood samples (K_2 EDTA tube, 9 mL) were taken at baseline and 6, 9, 12, 24, 36, 48, 60, 72, 78, 84, 96, 120, 144, 168, 192 and 216 h after application of the transdermal drugs. This was done to measure the plasma concentration of buprenorphine, its active metabolite norbuprenorphine and fentanyl (which has no active metabolites).

Pain assessment

For pain models involving pressure stimulation, the stimulation was carried out by the same investigator, as this previously has shown to improve consistency (Vatine *et al.*, 1993).

Superficial and deep pain models

Bone pressure stimulation. On the right tibia 15 cm below the patella a site was marked and used at every stimulation time. At this site, pressure stimulation was performed with a hand-held algometer (Type 2, Somedic Production AB, Sollentuna, Sweden) with probe size 2 mm in diameter. The rate of increase of force was 30 $kPas^{-1}$ adjusted to a probe size of 1 cm^2. The subjects were instructed to press a button when they reached the pressure tolerance threshold (PTT) and stimulation stopped. This stimulation has previously been demonstrated to be reproducible and to mimic bone pain evoked from the periosteum (T. Andresen *et al.*, unpubl. data).

Heat stimulation. An area (9 cm^2) of the skin on the right volar forearm, 10 cm proximal from the wrist, was heated

with a computerized 'Thermo Tester' (TSA II NeuroSensory analyser, Medoc Ltd, Ramat Yishai, Israel). The temperature increased from a baseline of 32°C to a maximum of 52°C with a rate of 1°C·s^{-1}. The subjects were told to press a button when the heat tolerance threshold was reached. Three successive stimulations were performed and between each of the stimulations the temperature returned to baseline. The average of the three stimulations was computed and used for the data analysis. The method is based on the Peltier principle and has shown to be reliable and reproducible over time (Heldestad *et al.*, 2010).

Electrical stimulation. Two bipolar patch electrodes (Neuroline 720, REF: 72001-K/12, Ambu a/s, Denmark) were placed over the median nerve on the left volar forearm, 2 cm distal to the wrist. The distance between the two electrodes was 1 cm. The electrodes were connected to a stimulation device (Isolator Stimulator Noxi IES 230, SN23006, JNI Biomedical, Klarup, Denmark) which was used to control the electrical stimulation. The subjects were stimulated with single (train of 5 pulses at 200 Hz) and repeated (5 stimuli (each consisting of a train of 5 pulses) at 2 Hz) stimuli (Arendt-Nielsen *et al.*, 1997). Pain detection threshold (PDT) was assessed for both single and repeated stimuli in three successions. The mean of the three stimulations was multiplied by 1, 1.4, 1.6 and 1.8 and the subjects were stimulated with these intensities in a randomized order. The subjects scored their pain according to the defined VAS. Electrical stimulation on the surface of the skin has shown to be reproducible (Lecybyl *et al.*, 2010).

Inflammatory and hyperalgesic pain models

UVB light first degree burn injury. Before evoking first degree sunburn erythema with UVB light (UVB) irradiation, the minimal erythema dose (MED) was determined. MED is the amount of UVB irradiation needed to give an irritated red area and was determined according to the subject's skin type (Hoffmann and Schmelz, 1999). MED was measured on the lateral side of the thigh 20 cm distal to the iliac crest using a UVB lamp (Phillips PL01, Narrowband UVB, λ_{max} = 320 nm, Cosmedico, Germany). The time required to give an irritated red area was multiplied by 3 and used to evoke the first degree sunburn (Gustorff *et al.*, 2004b). MED was determined prior to study start to ensure that it would not influence the actual stimulation.

For the first-degree sunburn stimulation, the thigh was divided into three areas of 9 cm^2 on the anterior side 20 cm distal to the iliac crest. Each of the areas was exposed to UVB-light (290–320 nm) at different times [t = 0 (UVB1), t = 48 (UVB2) and t = 120 h (UVB3)] 24 h prior to measurement (Gustorff *et al.*, 2004a). The same stimuli intensity was used for all sessions. In the first period the right thigh was used, in the second the left and in the third period the right thigh was used again.

The first degree sunburn area was defined as the primary hyperalgesic area and the area surrounding it was defined as the secondary hyperalgesic area. Heat tolerance threshold and PTT (performed with the pressure algometer with probe size 1 cm^2 and force increase rate of 30 kPas^{-1}) was assessed in the primary hyperalgesic area. Secondary hyperalgesia was quantified with a calibrated von Frey nylon filament (Touch Test Sensory Evaluator Kit, von Frey size 5.46, Stoelting Europe, Dublin, Ireland). The stimulation with the von Frey filament started in normal skin away from the primary area and continued until the subjects reported a clear change in sensation. This was performed in eight radial directions. The borders from normal to sensitized skin were marked with a pen and drawn on a transparency film and the area was calculated (Trust, 1200 wireless tablet, Trust International BV, Dordrecht, the Netherlands) (Gustorff *et al.*, 2004a). Stimulation with the filament was performed before pressure and heat stimulation.

Nerve growth factor. Based on a previous study a single dose of 2.5 µg nerve growth factor (NGF) in 0.1 mL (product number: 800479, human β-nerve growth factor, 25 µg·mL^{-1}, Aalborg Hospital Pharmacy, Aalborg, Denmark) was injected into the extensor digitor longus muscle, 10 cm distal to the patella on the lateral side of the left leg (Svensson *et al.*, 2003). PTT to pressure algometry was assessed before injection and 24, 48, 72 and 144 h after injection.

Capsaicin. Three sites were marked on the volar surface of the left forearm. The first site was marked 8 cm, the second 10 cm and the third 12 cm proximal from the wrist. An intradermal injection of 100 µg capsaicin in 0.1 mL (Product number: 800644, Capsaicin 1 mg·mL^{-1}, Aalborg Hospital Pharmacy) was injected with a sterile syringe (Torebjork *et al.*, 1992). The first site was used before administration of the drugs (baseline), the second site 72 h after application of the drugs and the third site 144 h after application of the drugs. The area surrounding the injection site was defined as secondary hyperalgesic and allodynic area. Secondary hyperalgesia area was quantified with a von Frey filament (for details see methods above for UVB light first degree burn injury) and the area of allodynia was quantified with a soft brush in the same way as the secondary hyperalgesic area. The hyperalgesic and allodynic areas were quantified immediately after injection. Subjects remained supine during the injection and under the assessment of the secondary areas of hyperalgesia and allodynia. The model is valid for pharmacological profiling of analgesics (Hughes *et al.*, 2002).

Adverse effects

The subjects were asked to report four of the most frequently adverse effects (nausea, dizziness, drowsiness and local irritation (itching due to the patch) at baseline and at every pain stimulation time (24, 48, 72 and 144 h). They were asked to rate their adverse effects with either 1 – nothing, 2 – light feeling, 3 – moderate feeling and 4 – intolerable feeling. All other adverse effects reported spontaneously by the subjects were recorded and rated in the same way.

Statistics

All results are expressed as mean ± SEM if not otherwise stated. Statistical calculations were based on change in stimulus intensity relative to baseline, that is, data corrected for baseline, to take into account possible inter-individual differences in baseline recordings. This has previously been shown to play an important role in the reproducibility of pain models (Staahl *et al.*, 2006). The software package SigmaPlot

11.0 (Systat Software Inc., San Jose, CA, USA) was used. The two drugs were only compared within the first 72 h of treatment. At 144 h only buprenorphine was compared with placebo as the fentanyl patch last for 72 h. To evaluate the results achieved within the first 72 h, a two-way analysis of variance was applied with these factors: (i) drug; and (ii) time. For *post hoc* analysis, Tukey Kramer's test was used. For the results determined at 144 h a *t*-test was applied. To test whether there was a difference in the adverse effect profiles between the treatments a χ^2 test was used. A P value < 0.05 was considered significant.

Results

Out of the 22 subjects one chose not to complete the study due to a job offer distant from the site and one was excluded after the second period due to hospitalization for a reason with no relation to the study. None of the subjects experienced intolerable adverse effects and no serious adverse events were recorded. Table 2 summarizes all adverse effects.

Data for the pain stimulations performed during each treatment period are summarized in Table 3.

Superficial and deep pain models

Bone pressure stimulation. The average baseline for PTT was 6223.5 ± 47.9 $kPas^{-1}$ (recalculated to probe size 2 mm in diameter). Within the first 72 h of treatment the subjects had a significantly higher pressure PTT when treated with buprenorphine compared with placebo ($P = 0.007$), but there was no difference between the two opioids ($P = 0.6$). Fentanyl was not better than placebo attenuating bone pain ($P = 0.09$) (Figure 2). In the placebo arm there was a significant fall in bone PTT within the first 72 h ($P = 0.04$), indicating that the stimulation paradigm induced sensitization.

At 144 h buprenorphine showed analgesic effects against bone pressure pain when compared with placebo ($P = 0.05$).

Heat stimulation. The average baseline for heat pain tolerance threshold was 46.3 ± 0.2°C. In comparison with placebo both buprenorphine and fentanyl showed analgesic effects, that is, increased the heat PTT ($P < 0.001$). Buprenorphine was also superior to placebo at 144 h ($P = 0.03$) (Figure 3).

Electrical stimulation. The average baseline value for PDT was 5.6 ± 0.6 mA for single stimulation and 4.2 ± 0.3mA for repeated stimulation. At the pain threshold there was no significant difference between the treatments for either single or repeated stimulation (both $P > 0.1$). Furthermore, there was no differences between the treatments for the pain intensities: 1*PDT, 1.4*PDT, 1.6*PDT and 1.8*PDT (for all assessments: $P > 0.3$).

Inflammatory and hyperalgesic pain models

UVB light first degree sunburn injury.

Primary hyperalgesia. Average baseline values for the PTT was 791.8 ± 24.8 $kPas^{-1}$ and for the heat tolerance threshold was 47 ± 0.2°C. None of the subjects had blisters. Buprenorphine significantly attenuated PTT in the primary hyperalgesic area within the first 72 h compared with placebo ($P = 0.006$), but there was no difference between the opioids ($P = 0.1$). Fentanyl was not superior to placebo ($P = 0.4$) (Figure 4). None of the treatments showed analgesic effects in sensitivity to heat in the primary hyperalgesic area ($P = 0.4$).

Secondary hyperalgesia. Out of the 20 subjects, six did not have a secondary hyperalgesic area assessed with the von Frey

Table 2

Number of subjects reporting adverse events in each of the active treatment periods

Reported adverse events	Fentanyl 24 h	Fentanyl 48 h	Fentanyl 72 h	Buprenorphine 24 h	Buprenorphine 48 h	Buprenorphine 72 h	Buprenorphine 144 h
Local irritation*	13	14	14	6	16	15	13
Nausea	12	2	5	16	13	10	3
Pruritus	10	6	2	6	10	9	2
Dizziness	19	11	7	18	18	18	8
Drowsiness	15	10	8	15	15	14	11
Fatigue	2			1	1		
Insomnia				2			
Vomiting	4			6	3	1	1
Dysuria	2	2	1	4	5	5	3
Constipation	1	5		5	4	3	3
Headache		2	2		1	3	
Other	1			2	2		2
Total	79	52	39	81	88	78	46

*Local irritation refers to itching underneath and the area around the patch.

Table 3

Schematic overview of the data from the experimental pain models

Experimental models	Fentanyl BASE	24 h	48 h	72 h	Buprenorphine BASE	24 h	48 h	72 h	144 h	Placebo BASE	24 h	48 h	72 h	144 h
Superficial/deep pain														
Pressure bone ($kPas^{-1}$) (*)	5712.6 ± 443.2	5524.8 ± 438.9	5311.4 ± 265.5	5529.5 ± 357.7	5697.6 ± 239.4	5522.3 ± 275.8	5786.4 ± 330.7	5875.8 ± 239.8	5714.3 ± 407.3	6240.4 ± 634.2	5687.8 ± 425.8	5308.0 ± 351.7	4976.7 ± 315.8	5343.0 ± 427.6
Heat (°C) (**)	45.7 ± 0.5	46.9 ± 0.5	47.3 ± 0.4	46.6 ± 0.5	46.3 ± 0.6	46.6 ± 0.6	48.2 ± 0.4	47.5 ± 0.5	47.5 ± 0.4	46.6 ± 0.5	46.2 ± 0.5	46.9 ± 0.4	45.9 ± 0.5	46.7 ± 0.5
Electrical single (mA)	6.3 ± 1.3	5.6 ± 1.1	5.8 ± 1.2	5.9 ± 1.3	5.7 ± 1.1	6.2 ± 1.1	5.8 ± 1.1	6.4 ± 1.2	6.5 ± 1.2	4.4 ± 0.7	4.50.8	4.9 ± 0.8	5.1 ± 0.8	5.9 ± 0.9
Repeated (mA)	4.4 ± 0.9	4.2 ± 0.8	4.3 ± 0.8	4.3 ± 0.9	4.4 ± 0.8	3.9 ± 0.7	4.2 ± 0.7	4.4 ± 0.7	4.7 ± 0.7	3.5 ± 0.4	3.4 ± 0.5	4.0 ± 0.6	4.1 ± 0.6	4.4 ± 0.7
Hyperalgesia/ inflammation														
UVB1 pressure ($kPas^{-1}$) (*)	834.1 ± 80.8	864.2 ± 75.8			728.3 ± 56.8	814.5 ± 83.6				752.8 ± 53.8	760.9 ± 61.7			
Heat (°C)	46.9 ± 0.5	44.6 ± 0.6			47.4 ± 0.5	44.3 ± 0.6				47.6 ± 0.4	44.0 ± 0.6			
Sec.HA (cm^2)UVB2 pressure ($kPas^{-1}$) (*)Heat (°C)	818.8 ± 70.9 46.4 ± 0.6	47.1 ± 8.3		911.3 ± 84 44.9 ± 0.6	803.7 ± 67.7 46.8 ± 0.5	39.6 ± 5.6		1003.8 ± 101.7 45.4 ± 0.5		862.3 ± 67.1 47.3 ± 0.4	44.0 ± 6.7		755.7 ± 46.54 5.1 ± 0.7	
Sec.HA (cm^2)				34.0 ± 5.7				47.6 ± 9.6					50.73 ± 7.3	
UVB3 pressure ($kPas^{-1}$) (*)					690 ± 56.8				738.7 ± 72.7	695.5 ± 46.1				686.2 ± 54.9
Heat (°C)					47 ± 0.6				45.1 ± 0.5	46.5 ± 0.5				44.4 ± 0.7
Sec.HA (cm^2)									34.8 ± 5.3					37.42 ± 5.1
Pressure (NGF) ($kPas^{-1}$)	869.4 ± 64.1	769.1 ± 71.8	832.4 ± 79.9	886.6 ± 79.5	904.9 ± 98.3	733.4 ± 81.1	778.8 ± 88.2	827.4 ± 84.5	907.0 ± 90.9	867.6 ± 72.9	674.2 ± 71.6	707.2 ± 68	763.5 ± 84.1	913.9 ± 95
Capsaicin Sec.HA (cm^2)	51.1 ± 6.8			55.2 ± 8.1	50.3 ± 10.2			58.6 ± 9.5	53.87.5	53.9 ± 10.9			59.5 ± 10.9	48.1 ± 9.4
Allodynia (cm^2)	41.5 ± 7.2			45.1 ± 8.1	38.5 ± 6.1			47.6 ± 8.3	38.07.4	40.5 ± 8			43.7 ± 8.1	52.8 ± 7.7

*Significant effect of buprenorphine to bone associated pain and to pressure in the primary hyperalgesic area induced with UVB-light compared with placebo.

**Significant effect of buprenorphine and fentanyl to cutaneous heat pain.

Data are shown as mean ± SEM.

Allodynia refers to area assessed by brush stimulation.

BASE, baseline recordings; CP, cold pressor; AUC, area under the curve; VAS, visual analogue scale; UVB, ultra violet B-light (1, 2 and 3 refers to the different stimulation areas used in each treatment period); Sec.HA, secondary hyperalgesia assessed by pinprick; NGF, nerve growth factor.

Statistical comparisons of the effect of buprenorphine and fentanyl to the different pain tests within the first 72 h were investigated using two-way analysis of variance. For the investigation of the effect, data where analysed as a relative change compared with baseline.

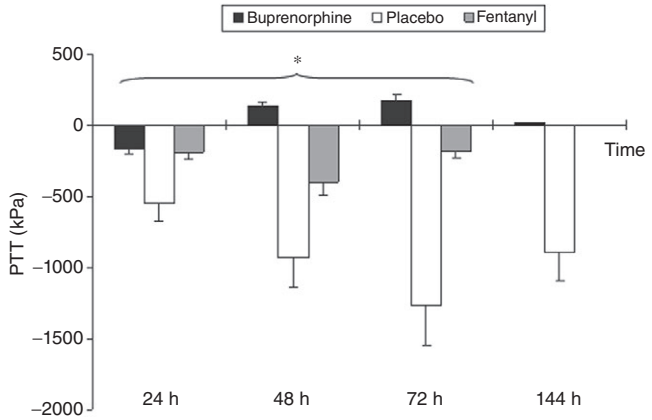

Figure 2

The figure illustrates the results from pain assessed by pressure at the tibial bone. Pressure was applied before and 24, 48, 72 and 144 h after application of the patches. Data shown represent the difference in PTT between baseline values and those at 24, 48, 72 and 144 h after application of the patches (mean ± SEM). The two-way ANOVA revealed an overall effect within the first 72 h of treatment where buprenorphine was superior to placebo to pressure at the tibia (*P = 0.007), whereas fentanyl was not significantly different from placebo (P = 0.09). At 144 h buprenorphine showed analgesic effect against bone pressure pain, compared with placebo (P = 0.05).

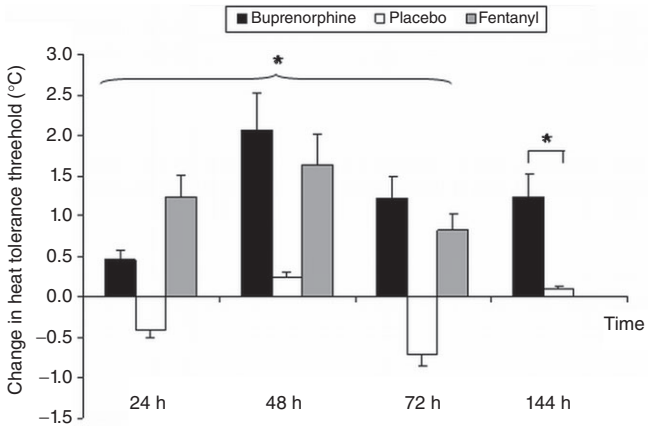

Figure 3

The figure shows the results from the cutaneous heat stimulation model. Heat tolerance thresholds were determined before and 24, 48, 72 and 144 h after application of the patches. Data shown represent the difference in heat tolerance threshold between baseline values and those at 24, 48, 72 and 144 h after application of the patches (mean ± SEM). Analysis by two-way ANOVA showed an overall analgesic effect of both drugs to heat within the first 72 h, when compared with placebo (*P < 0.001). In addition, buprenorphine significantly increased heat tolerance threshold at 144 h compared with placebo (P = 0.03) (*). Data are illustrated as change in heat tolerance threshold from baseline to 24, 48, 72 and 144 h after application of the patches (mean ± SEM).

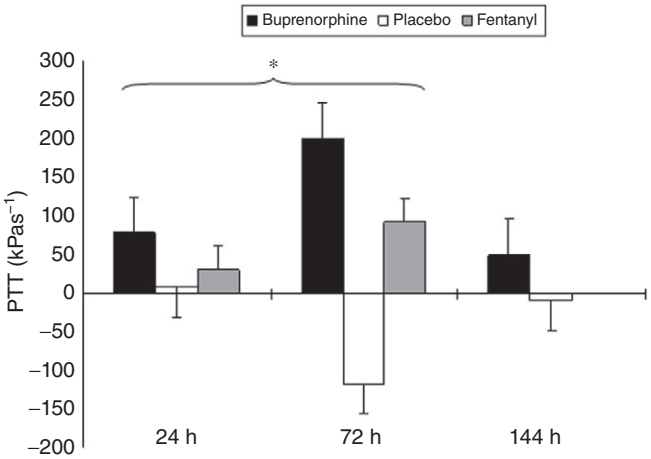

Figure 4

The figure shows the pressure PTT in the primary hyperalgesic area induced by UVB light. Measurements were performed before and 24, 72 and 144 h after application of the patches. Data shown represent the difference in PTT between baseline values and those at 24, 48, 72 and 144 h after application of the patches (mean ± SEM). Analysis by two-way ANOVA revealed an overall effect within the first 72 h where buprenorphine significantly attenuated PTT compared with placebo (*P = 0.006) but not when compared with fentanyl. Fentanyl was not superior to placebo (P = 0.1). No differences between buprenorphine and placebo were seen at 144 h (P = 0.4).

filament. There was no reduction in the secondary hyperalgesic area assessed with pinprick for any of the drugs (P > 0.8).

At 144 h there was no difference between buprenorphine and placebo in PTT (P = 0.3) or sensitivity to heat in the primary hyperalgesic area (P = 0.7), and no change in the secondary hyperalgesic area to pinprick (P = 0.5).

Nerve growth factor. The average baseline for PTT was 900.7 ± 23.3 $kPas^{-1}$. There was no analgesic effects for any of the drugs at any time to NGF induced sensitization in the muscle (all P > 0.2).

Capsaicin. The average baseline for the secondary hyperalgesic area assessed with von Frey filament was 50.8 ± 1.6 cm^2 and for the allodynic area assessed with brush it was 38.6 ± 1 cm^2. There was no significant analgesic effect of the drugs at any time in the pin-prick areas (P > 0.9) or the allodynic areas (P > 0.7).

Adverse effects

The most frequent adverse effects experienced by the subjects were nausea, dizziness, drowsiness and local irritation (itching due to the patch) throughout each of the active treatment periods. None of the subjects reported a score of 4 (intolerable feeling) for any of the adverse effects.

There was no significant difference in the frequency of adverse effects between buprenorphine and fentanyl (P = 0.1). However, regarding severity of the adverse effects, a difference was observed at 48 h for dizziness, where the score was higher for buprenorphine than fentanyl (P = 0.03).

After 144 h there was a higher recording of dizziness (P = 0.004) and drowsiness (P < 0.0001) for buprenorphine when compared with placebo. The registration of nausea and local irritation (itching due to the patch) was not different between buprenorphine and placebo (P = 0.3).

Discussion

This experimental study investigated tissue and modality specific analgesic and anti-hyperalgesic effects of buprenorphine and fentanyl. Buprenorphine attenuated bone associated pain as well as mechanical stimulation in the UVB-induced primary hyperalgesic area when compared with placebo, which was not the case for fentanyl. Both active drugs attenuated the heat pain tolerance threshold. Neither of the opioids attenuated NGF-induced muscle soreness or capsaicin-induced hyperalgesia.

Methodology

The experimental pain models used in the present study are well-known methods, which have been demonstrated to be reproducible and valid. Superficial and deep pain models as well as models inducing hyperalgesia have shown sensitivity towards opioids (Staahl *et al.*, 2009). The current experimental approach using superficial and deep pain models provided an opportunity to investigate tissue and modality differentiated effects of opioids. On the other hand, experimental hyperalgesic models can act as proxies for clinical manifestations and are more clinical relevant than phasic pain models (Negus *et al.*, 2006). Nevertheless, it is important to keep in mind that models inducing hyperalgesia are more difficult to control with respect to reproducibility compared with the phasic pain models (Staahl *et al.*, 2009).

In the present study only a single dose was used, which may be the reason why it was not possible to reproduce the clinical situation adequately. Several studies have shown analgesic/anti-hyperalgesic effects of single-dose opioids against different experimental pain stimuli (Staahl *et al.*, 2009), but conflicting results have been observed using electrical stimulation and intradermal injection of capsaicin to induced hyperalgesia (Staahl *et al.*, 2009). Hence, these models (as well as the NGF-muscle induced soreness) may be less suitable to demonstrate the effect of analgesics.

A study by Koltzenburg *et al.* showed analgesic effects with transdermal patches providing 25 $\mu g \cdot h^{-1}$ fentanyl and 35 $\mu g \cdot h^{-1}$ buprenorphine (Koltzenburg *et al.*, 2006). Sittl *et al.* suggested that the analgesic potency-ratio to morphine would be 1:110 to 1:115 for buprenorphine and 1:100 for fentanyl (Sittl *et al.*, 2005). In the present study, the analgesic and anti-hyperalgesic effects of the buprenorphine patch 'Norspan®' with a treatment duration of 144 h was investigated. Based on the assumptions of Sittl *et al.* patches releasing 25 $\mu g \cdot h^{-1}$ fentanyl (72 h treatment) and patches releasing 20 $\mu g \cdot h^{-1}$ buprenorphine (144 h treatment) were chosen (Sittl *et al.*, 2005). Nevertheless, this leads to a difference in duration of time to reach maximum plasma concentration as this is reached after 24 to 36 h for buprenorphine and 12–24 h for fentanyl (Grond *et al.*, 2000; Johnson *et al.* 2005). The effects of the drugs were therefore compared within the first 72 h as an overal. At 144 h buprenorphine was only compared with placebo.

An issue with μ-opioid agonists is dependence and tolerance. However, there are several factors, for example, genetic variation, drug formulation and drug administration that have an influence on why individuals differ in their responses to addictive drugs (Ballantyne and LaForge, 2007). In the current study, it was highly unlikely that dependence and tolerance played a role as only a single dose was investigated and thus dose escalation to produce previously attained levels of response is not possible.

A drug with multiple effects at the opioid receptors would have been preferable to use as a comparator in this study. Unfortunately no comparators with efficacy at multiple opioid receptors exist and to have comparable administration forms, fentanyl was chosen. In addition, comparing the multiple mechanisms of buprenorphine to a pure μ-opioid agonist such as fentanyl is of major interest. On the other hand, the many ancillary opioid actions of buprenorphine and its metabolite can complicate the interpretation of results.

Superficial and deep pain models

Based on a previous study by Koltzenburg *et al.* (2006) investigating the effects of transdermal opioids to experimental pain, we used the same drugs and mode of administration in the present study (Koltzenburg *et al.*, 2006). However, they used a treatment regimen of 72 h for fentanyl and 96 h for buprenorphine, whereas in our study the duration of treatment was 144 h for buprenorphine. Koltzenburg *et al.* showed analgesic effects to the cold pressor test, but failed to show a significant effect to heat stimulation for both drugs. It is well known that opioids are sensitive to both heat stimuli and the cold pressor test (Staahl *et al.*, 2009). In the present study both drugs significantly increased the heat pain tolerance threshold. The difference between our study and the study by Koltzenburg *et al.* may be related to methodological issues.

The pathogenesis of bone associated pain is still not fully understood, but it is known that the periosteum is innervated by unmyelinated nociceptive afferents (Gronblad *et al.*, 1984). These nociceptors are sensitive to high intensity pressure (Gronblad *et al.*, 1984; Rosier 1992), and animal studies have indicated that δ-opioid receptors may play an important role in controlling bone associated nociception (Mizoguchi *et al.* 2003; Brainin-Mattos *et al.*, 2006). It is, however, difficult to measure and treat this kind of pain (Delaney *et al.*, 2008). Clinically, much is known about manifestation of bone-related pain from the study of metastatic bone cancers, where the nociceptive and neuropathic components are well described (Mercadante, 1997). The greatest component of nociception is likely to relate to the periosteum as Kellgren showed that no pain was felt by drilling a wire through the upper part of the tibia after anesthetizing the overlying skin and periosteum (Kellgren, 1939). Experimental pressure on the tibia in healthy volunteers was shown to evoke bone associated pain and mimic the clinical situation (Hamilton *et al.*, 1967; Vatine *et al.* 1993). In the placebo arm, a decrease in PTT over time was observed. This was most likely due to hyperalgesia developed as a consequence of repeated pressure at the tibia. Pressure to the tibia displayed the tissue-differentiated effects of the two opioids. Here, buprenorphine

attenuated the pain manifested as an anti-hyperalgesic effect, most likely via norbuprenorphine, as this metabolite is a strong δ-opioid receptor agonist (Huang *et al*., 2001; Kress 2009). The contribution of norbuprenorphine to the analgesic effect of buprenorphine is likely, as norbuprenorphine was measurable in the plasma (data on file, but not available). Fentanyl did not attenuate the pressure-induced hyperalgesic pain. This could be due to the fact that δ-receptors play an important role in hyperalgesic conditions (Negus *et al*., 1989; Brainin-Mattos *et al*., 2006; Gaveriaux-Ruff *et al*. 2008) and a pure μ-receptor agonist like fentanyl might therefore not be beneficial in treating such pain conditions.

Electrical stimulation activates several nerve fibres in the skin and sends strong noxious signals to the CNS (Staahl and Drewes, 2004). A number of studies have shown analgesic effects of opioids to cutaneous electrical stimulation (Naef *et al*., 2003; Staahl *et al*. 2006). Repeated electrical stimulation which was also used in the current study evokes an integration of pain, which especially is thought to be well modulated by opioids (Brennum *et al*., 1993; Schulte *et al*. 2003). However, the present study failed to show an analgesic effect to any of the electrical stimulation paradigms, which was also the case for the study conducted by Koltzenburg *et al*. In the present study, habituation was observed for PDT for both single and repeated electrical stimulation in the placebo arm which could explain the lack of effect. Furthermore the PDT was determined which might result in less C-fibre activation, compared with the measurement of the PTT (Brennum *et al*., 1993). Even stimulation with 1.8*PDT resulted in average VAS below or at the tolerance threshold.

Inflammatory and hyperalgesic pain models

Chronic pain is often associated with peripheral or central hypersensitivity conditions (Curatolo *et al*., 2006). Secondary hyperalgesia is mainly considered a manifestation of hypersensitivity of the CNS and is often seen in patients with chronic pain (Curatolo *et al*., 2006; Taylor 2009). Animal studies suggest that δ-opioid receptor agonists show anti-hyperalgesic effects (Fraser *et al*., 2000), and that dynorphin, an endogenous κ-opioid receptor agonist, promoted hyperalgesic pain states (Vanderah *et al*., 2000). Buprenorphine might counteract hyperalgesia by its antagonistic properties at κ-opioid receptors (Brainin-Mattos *et al*., 2006; Pergolizzi *et al*. 2010). Animal studies may provide pharmacodynamic information, but have limitations mimicking human pain conditions. Clinical trials with patients offer a way to explore the actual pain states of interest. On the other hand, human experimental pain models overcome the species gap (Arendt-Nielsen *et al*., 2007). This was demonstrated in previous studies from our laboratory showing tissue-differentiated effects of opioids, reflecting the clinical situation (Staahl *et al*. 2006; Olesen *et al*., 2010). In humans experimental pain models using irradiation with UVB light, injection of NGF or capsaicin have been used to induce tissue-specific primary and secondary hyperalgesia, which makes them valuable in studies investigating anti-hyperalgesic effects of analgesics (Simone *et al*., 1989; Svensson *et al*. 2003; Sycha *et al*., 2003). Our study used these models to see if buprenorphine also produces anti-hyperalgesic effects in types of inflammation other than that used in the model by Koppert *et al*.

Numerous studies have demonstrated sensitivity of analgesics in the primary hyperalgesic area induced by UVB irradiation (Bickel *et al*., 1998; Sycha *et al*., 2003; Gustorff *et al*. 2004b). A previous study found better analgesic effects of opioids to heat at the perception threshold, compared with the tolerance threshold, when applied in the primary hyperalgesic area (Gustorff *et al*., 2004b). It is not known whether these differences between perception and tolerance thresholds could result from different spinal and supraspinal sites of sensitization and/or mechanisms of opioid responsiveness (Gustorff *et al*., 2004b). Nevertheless, in the present study it was not possible to show analgesic effects to heat tolerance threshold in the primary hyperalgesic area. A recent study performed by Bishop *et al*. showed sensitivity to mechanical stimulation in the primary lesion site (Bishop *et al*., 2009). In the present study, buprenorphine significantly increased pressure PTT in the primary lesion site compared with placebo. This measures the peripheral analgesic effect of the drug as primary hyperalgesia reflects the peripheral sensitization of nociceptors (Gustorff *et al*., 2004b). Nevertheless, a central effect cannot be excluded as a study by Draxler *et al*. could not show an effect of topical buprenorphine against UVB-induced primary hyperalgesia (Draxler *et al*., 2008).

Gustorff *et al*. showed that the opioid remifentanil reduced the UVB-induced secondary hyperalgesic area (Gustorff *et al*., 2004b), but we could not reproduce these findings with fentanyl and buprenorphine. Nevertheless, the set-up and size of the von Frey filaments was different between these studies and hence the results are not directly comparable.

Intramuscular injection of NGF in healthy volunteers has been shown to induce long-lasting, pressure-evoked, soreness of the muscle and time-dependent pressure hyperalgesia, which most likely involve both central and peripheral mechanisms (Svensson *et al*. 2003; Andersen *et al*., 2008). As in previous studies (Svensson *et al*. 2003; Andersen *et al*., 2008) we found NGF induced sensitization in the placebo arm within the first 72 h after injection and was most prominent after 24 h. In our study it was not possible to show an analgesic effect to pressure after intramuscular injection of NGF even though a sensitization was present. Animal studies suggest that NGF interferes with nociceptive neurons and attenuates the analgesic effects of opioids (McDowell, 2004; Mousa *et al*. 2007), which could have influenced the analgesic effects of the drugs in the present study.

Intradermal injection of capsaicin is a valid model for testing central anti-hyperalgesic effects of analgesics (Scanlon *et al*., 2006). The model mainly induces secondary hyperalgesia (Simone *et al*., 1989) and doses around 100 μg capsaicin are normally used (Torebjork *et al*. 1992; Gottrup *et al*., 2004). In the present study no anti-hyperalgesic effects of the drugs to capsaicin-induced hyperalgesia could be shown. Previous studies have shown conflicting results regarding the anti-hyperalgesic effect of opioids against the secondary hyperalgesic area when assessed by pin-prick. Anti-hyperalgesic effects of alfentanil to pin-prick hyperalgesia induced by intradermal capsaicin (Eisenach *et al*., 1997; Wallace *et al*. 2002) was not confirmed by Sethna *et al*. (Sethna *et al*. 1998).

Clinical aspects

Both transdermal buprenorphine and fentanyl are currently used in the treatment of non-malignant and malignant chronic pain conditions.

Non-malignant pain. In the treatment of chronic non-malignant pain clinical studies have shown that a 7 day dosing regimen of transdermal buprenorphine reduced the pain intensity in patients with chronic low back pain and osteoarthritis (Johnson *et al*. 2005; Gordon *et al*., 2010). Several other clinical studies have shown analgesic effects of buprenorphine against post-operative pain (Dobkin *et al*., 1977; Kay, 1978; Johnson *et al*. 2005). In contrast, transdermal fentanyl is contraindicated in postoperative pain due to the increased risk of respiratory complications. Nevertheless, fentanyl has shown to be valuable in the treatment of mild to severe chronic pain (Park *et al*., 2011).

Malignant pain. Transdermal buprenorphine has also been studied for the treatment of chronic cancer pain (Johnson *et al*., 2005; Pergolizzi *et al*. 2010). However, most of these studies represent small and uncontrolled trials, and there is therefore a need for further data to support the use of transdermal buprenorphine for the treatment of cancer-related pain (Johnson *et al*., 2005). On the other hand, transdermal fentanyl has been used widely in the treatment of pain associated with cancer (Jeal and Benfield, 1997). A clinical study showed that there were no differences in analgesia between buprenorphine and fentanyl when patients were switched between opioids (Aurilio *et al*., 2009). However, to our knowledge there are no clinical studies showing analgesic differences between buprenorphine and fentanyl against bone-associated pain.

In conclusion, our study showed modality- and tissue-differentiated effects of buprenorphine and fentanyl. Buprenorphine had a significant analgesic effect against experimentally induced bone-associated pain and primary hyperalgesia when compared with placebo, which was not the case for fentanyl. This may reflect observations in the clinic, where some opioids are more effective than others in individual patients.

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Conflict of interest

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