Effect of peripheral morphine in a human model of acute inflammatory pain

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Several studies have demonstrated the presence of opioid inducible receptors on peripheral nerves and peripheral antinociceptive effects of opioids. However, the effects of peripheral opioid administration in man are controversial. Our study used a randomized, double-blind, placebo-controlled, three-way crossover design in a human model of acute inflammatory pain (heat injury). We studied 18 healthy volunteers who each received morphine locally (2 mg), morphine systemically (2 mg), or placebo on three separate study days. The subjects received morphine infiltration subcutaneously (s.c.). 1 h before heat injury (47°C, 7 min) and naloxone infiltration s.c. (0.2 mg) 2.5 h after the heat injury. Hyperalgesia to mechanical and heat stimuli were examined using von Frey hairs and thermodes, and pain was rated using a visual analogue scale. The burns produced significant hyperalgesia, but local morphine infiltration neither reduced pain during the burn, nor primary or secondary hyperalgesia to mechanical and heat stimuli after the burn. In conclusion, peripherally applied morphine had no acute antinociceptive effects in this human model of acute inflammatory pain.

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The presence of opioid receptors has been demonstrated on peripheral terminals of thinly myelinated and unmyelinated nerves as well as on lymphocytes.1–4 Inflammation may upregulate peripheral opioid receptors and the density appears to increase minutes to hours after an inflammatory reaction is initiated.1–2 These findings suggest that opioids may have peripheral analgesic effects.

The analgesic effect of intra-articular opioid administration in the knee cavity has been studied extensively, and it has been concluded that intra-articular morphine may have some effect in reducing postoperative pain intensity, but final conclusions were hindered by flaws in designs and samples sizes of the studies.5 Peripheral analgesic effects of opioids applied at extra-articular sites have been difficult to demonstrate, and a recent review concluded on the basis of 26 trials with data from 952 patients that there was no evidence for a clinically relevant peripheral analgesic effect of opioids in acute extra-articular pain.6

However, in the view of the clinical potential, there is a need for further human studies investigating the analgesic effects of peripheral morphine. The aim of our study was to examine the antinociceptive effect of morphine infiltrated subcutaneously (s.c.) in a human model of acute inflammatory pain. One trial has previously tested morphine in a similar burn model, but with limited sensory assessments and without systemic morphine as control.7

Methods

We studied 18 healthy volunteers (16 men) who were aged 22–48 yr. Informed consent was obtained from all subjects and the protocol was approved by the Municipal Ethical Committee of Copenhagen. During a pre-study training session, they were interviewed about their health history and were given a physical examination. In this session the volunteers experienced the burn and trained all assessments performed during the study.

The study used a randomized, double-blind, placebo-controlled, three-way crossover design. Each volunteer received either morphine (2 mg, 5 ml) s.c. in the burn injury area (local), morphine in the corresponding area on the opposite leg (systemic), or saline (0.9%, 5 ml) in both legs (placebo) on three study days separated by 2 weeks. When subjects received morphine, they received saline in the opposite leg. The order of the study days was randomized en bloc, so the treatments were equally distributed over the
three study days. Morphine or placebo (saline) was injected after baseline measurements and measurements were repeated thereafter. The burn was induced on the non-dominant leg 1 h after the injections, and measurements were made at 0, 1 and 2 h after the burn. Subjects received naltrexone (0.2 mg, 5 ml) in both areas, 2 h and 25 min after the burn injury, and measurements were repeated 30 and 90 min after the naltrexone injections. The order and duration of the different measurements has been described previously. All testing was performed by two experimenters in a quiet room at a temperature of 23–26°C. All subjects were tested by the same experimenter at the same time of the day on the three study days. The subjects were resting in a relaxed position and instructed to keep their eyes closed during all measurements.

All injections were given using a needle, 40 mm long with an external diameter of 0.4 mm. Morphine (SAD, Copenhagen, Denmark) was used at the concentration of 0.4 mg ml⁻¹ (pH 4.1), naltrexone (Du Pont Pharma, Meda AS, Copenhagen, Denmark) at the concentration of 0.04 mg ml⁻¹ (pH 4.3). In all injections the volume was 5 ml. The injections were given from opposite corners of the 50×25-mm drawn rectangles, 2.5 ml from each corner, injected in a fan-like manner, in order to distribute the volume evenly in the rectangle. The injections were made in the most superficial part of the s.c. tissue. A person not involved in the testing administered the injections and prepared the morphine and placebo. Subjects were asked to evaluate the pain associated with each injection, using a visual analogue scale (VAS 0–100).

Burns were produced on the medial part of the non-dominant distal leg with a 50×25-mm thermode (Thermotest, Somedic A/B, Stockholm, Sweden). The burns were induced in the same area on the three study days. The thermode (47°C) was applied to the skin for 7 min under standardized pressure (6.9 kPa). This caused redness without blistering.

The pain was rated with a VAS at the start and every minute during the burn injury. The scale was anchored by the descriptors, ‘no pain’ (0) and ‘worst pain imaginable’ (100). Verbal descriptors were added at 2 mm (weak pain), 8 mm (mild pain), 18 mm (moderate pain), 39 mm (intense pain) and 74 mm (very intense pain) to make the scale more comprehensible. The subjects rated the pain with a slider on a plastic device that was pulled along a 10 cm line between the end points. The numeric value of the rating was read on the back of the device by the experimenter.

The mechanical pain threshold within the injury area was determined by pinprick with nine progressively rigid von Frey hairs (Senselab Aesthesiometer, Somedic A/B, Stockholm, Sweden) numbered one–nine (force of hairs: hair 1=6 mN, 2=9 mN, 3=17 mN, 4=27 mN, 5=46 mN, 6=77 mN, 7=118 mN, 8=228 mN, 9=314 mN). Suprathreshold pain responses to mechanical stimuli were assessed using the VAS following stimuli with a von Frey hair (314 mN). The area of mechanical hyperalgesia to punctate stimuli that developed around the burn area was assessed using a rigid von Frey hair (428 mN).

Thermal thresholds were determined using a computerized contact thermode (Somedic A/B, Stockholm, Sweden). All thresholds were assessed using a 25×50-mm thermode, and determined as the average of three trials performed at 9-s intervals, from a baseline temperature of 32°C, and with a rate of change of 1°C s⁻¹. The upper cut-off limit was 52°C. The pain response (VAS 0–100) to heat was evaluated with a heat stimulus (15×25-mm thermode) of 45°C lasting 5 s, preceded by a temperature rise from 40 to 45°C over 5 s.

The subjects completed a questionnaire at the end of each day concerning the presence (yes/no responses) of euphoria, nausea, bluntness, headache, dizziness, confusion, feeling drunk, itching and restlessness. When side effects were present, these were rated as weak, moderate or marked.

Normality of raw data and differences between groups were evaluated using the Shapiro–Wilk’s W-test. Data are presented as means or medians dependent on the distribution (normal or skewed), and comparisons were made using the Student’s t-test for a paired design, when the differences showed normal distributions, whereas differences showing non-normal distributions were analysed using the Wilcoxon matched-pairs test. If baseline assessments differed significantly between groups, changes from baseline assessments were compared. Comparisons of more than two groups, such as changes over time, were performed using parametric analysis of variance (ANOVA) for repeated measurements (one-way), or the Friedman ANOVA, as appropriate. The smallest differences between placebo and local morphine detectable with a power of 80% and a type I error of 5% were calculated for all measurements at all time points. P-values below 0.05 were considered statistically significant.

Results

Baseline assessments did not differ significantly except for heat pain detection thresholds. Because thresholds were higher for placebo than for systemic treatment (P=0.01), and higher for systemic than for local treatment (P=0.03), further comparisons between treatments for heat pain detection thresholds were performed using changes from baseline assessments. Post-drug assessments (performed pre-burn) differed significantly for two measurements: mechanical pain outside the burn area was higher for local than for systemic treatment (P=0.01); and the pain response to heat (45°C, 5 s) was more intense for local than for placebo treatment (P=0.04). The pain response (VAS 0–100) to the burn was not significantly altered by either systemic or local morphine treatment (Fig. 1). In 11 out of 18 subjects, allodynia developed around the burn injury area when tested 4–6 min after start of the burn. The areas of allodynia were larger with local than systemic treatment (P=0.04). Neither local nor systemic morphine treatment
changed the areas of alldynia significantly compared with placebo.

The burns decreased mechanical pain thresholds ($P<10^{-5}$) and increased pain responses to mechanical stimuli within the site of injury ($P<10^{-6}$, Fig. 2A and B). Neither the pain threshold nor the suprathreshold pain response was significantly altered by local morphine compared with systemic morphine or placebo. The burn produced significant areas of hyperalgesia to punctate mechanical stimuli around the injury ($P<10^{-5}$), and the burns increased the pain responses to mechanical stimuli in the area of secondary hyperalgesia ($P<10^{-5}$, Fig. 3A and B). Burns treated with local morphine developed larger areas of secondary hyperalgesia than when treated with systemic morphine 0–2 h after the burn injury ($P=0.03$). After naloxone injection, burns treated with local morphine showed larger areas of secondary hyperalgesia than burns treated with systemic morphine or placebo ($P=0.04$). Pain responses to mechanical stimuli in the area of secondary hyperalgesia showed no significant differences between local, systemic or placebo treatment.

Pain responses to 45°C (5 s) were increased by the burn ($P<10^{-5}$), and heat pain thresholds were decreased in the burn area when treated with placebo or systemic morphine ($P<0.04$), whereas thresholds were not significantly affected by the burn when treated with local morphine. However, the heat pain threshold was not significantly increased by local morphine compared with systemic morphine or placebo, nor was the pain during the brief (5 s) heat stimuli reduced by local morphine compared with systemic morphine or placebo (Fig. 4A and B).

The burn induced an increase in warm detection thresholds for all treatments ($P<10^{-5}$, Fig. 5A). Cold detection thresholds were only significantly increased by the burn, when treated with local morphine ($P=0.007$, Fig. 5B).
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**Fig 4** Pain response to a heat stimulus of 45°C (medians, A) and heat pain thresholds (means, B). The pain during the brief (5 s) heat stimuli was not reduced by local morphine, nor was the heat pain threshold significantly increased by local morphine compared with systemic morphine or placebo.

**Fig 5** Warm detection thresholds (means, A) and cold detection thresholds (medians, B). Neither warm nor cold detection showed significant differences between treatments 0–2 h after the burn injury. After naloxone injections both warm and cold detection thresholds were significantly higher with systemic morphine compared with placebo ($P<0.03$). None of the other treatments differed significantly.

<table>
<thead>
<tr>
<th>Pain during burn</th>
<th>Warm detection threshold ($^\circ$C)</th>
<th>Cold detection threshold ($^\circ$C)</th>
<th>Heat pain threshold ($^\circ$C)</th>
<th>Heat pain response to 45°C for 5 s</th>
<th>Mechanical pain in burn area (VAS: 0–100)</th>
<th>Mechanical pain threshold (VAS: 0–100) (von Frey no)</th>
<th>Area of secondary hyperalgesia (cm²)</th>
<th>Mechanical pain in zone of secondary hyperalgesia (VAS: 0–100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(VAS: 0–100)</td>
<td>6.6</td>
<td>0.9</td>
<td>0.8</td>
<td>1.9</td>
<td>3.9</td>
<td>0.9</td>
<td>15.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Neither warm nor cold detection showed significant differences between treatments before or 0–2 h after the burn injury.

Subjects evaluated the pain caused by the injection using a VAS-score. Saline injections were rated with a mean score on the VAS of 31, morphine with a mean score of 35 and naloxone with a mean score of 41. Pain response to injection was significantly more intense for morphine than for saline injections ($P<0.02$).

All side effects (euphoria, nausea, bluntness, headache, dizziness, confusion, feeling of being drunk, itching and restlessness) were reported except headache. However, most side effects were reported by only one–three subjects out of 18 and were rated as weak. One exception was itching which was reported by seven subjects of which two rated the intensity of itching as moderate and one rated it as marked.

When saline was injected only one subject reported dizziness. No other side effects related to saline were reported.

The smallest differences between placebo and local morphine detectable at single time points are presented in Table 1. The values are the means of seven observation times (baseline, post-drug, 0, 1 and 2 h after heat injury, and 30 and 90 min after naloxone injection).

**Discussion**

We examined the antinociceptive effect of morphine infiltrated s.c. in a human model of acute inflammatory pain based on a standardized burn injury in healthy volunteers. Our results suggest no antinociceptive effect of morphine, as none of our assessments showed signifi-
cantly decreased pain intensity or increased pain thresholds when treated with local morphine compared with systemic morphine or placebo. In contrast, local morphine may have contributed to hyperalgesia as the areas of secondary hyperalgesia were larger when treated with local morphine compared with systemic morphine. The finding that local naloxone did not change hyperalgesia supports the lack of a peripheral analgesic effect of morphine. Further, we were not able to detect any analgesic action of the low dose of morphine (2 mg) as none of the pain assessments was significantly different between placebo and systemic morphine, and side effects rated as weak were only observed in one–three of 18 subjects. Only warm detection thresholds increased when treated with systemic morphine compared with placebo. In summary, peripheral morphine administration did not reduce pain significantly either in normal skin or in hyperalgesic skin.

Our results may have been influenced by the pH of the morphine solution. The pH of morphine was 4.1, naloxone 4.3 and saline 5.6. Injection of morphine induced a significantly higher pain score compared with saline injections (P<0.02), which may be explained by the lower pH. The acidic morphine solution may be local irritating and increased sensitization of nociceptors in the burn area, thereby counteracting the possible antinociceptive effect of morphine. However, the morphine solution used was the one generally applied for clinical use. Further, we cannot exclude a minor analgesic effect of morphine, as we were able to detect an ~40% difference between the overall mean of measurements between treatments and with a power of 80% for most measurements.

Our results are not contradictory to the clinical studies wherein extra-articular administration of opioid had no relevant effect,6 or the studies of intra-articular administration which have shown variable effects.5 However, our results do not exclude a late (e.g. >8 h) analgesic effect of peripheral morphine.10 In other experimental human pain models (capsaicin,11 thermal stimulation,12 propofol injection pain13), the peripheral analgesic effects of opioids have also been debatable, as only the capsaicin model showed a peripheral analgesic effect.11

In conclusion, our results cannot verify acute antinociceptive effects of peripheral morphine in this model of acute inflammatory pain.

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References