Increased numbers of opioid expressing inflammatory cells do not affect intra-articular morphine analgesia

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Background. Both locally expressed β-endorphin (END) and low doses of morphine relieve pain within inflamed knee joints. Here we examined whether enhanced inflammation and END expression within the synovial tissue of patients undergoing arthroscopic knee surgery might shift the analgesic dose–response curve of intra-articular (i.a.) morphine.

Methods. Following IRB approval and informed consent, patients were randomly assigned to the following i.a. treatments at the end of surgery: group I (n=39), isotonic saline; group II (n=40), 1 mg morphine hydrochloride; group III (n=48), 2 mg morphine hydrochloride; group IV (n=39), 4 mg morphine hydrochloride. Postoperative pain intensity was assessed by the visual analogue scale (VAS), by the time to first analgesic request and by the supplemental piritramide consumption. Synovial specimens from each patient were stained for the presence of inflammatory cells and END and were discriminated into groups with low versus high numbers of these cells. Differences between groups were statistically analyzed by χ², ANOVA and MANCOVA where appropriate.

Results. Patient characteristics and VAS scores did not differ between groups. Total postoperative piritramide consumption decreased and the time to first analgesic request increased significantly with increasing doses of i.a. morphine (P<0.05, ANOVA and linear regression). These dose–response relationships were not different between patients with low versus high numbers of inflammatory and END-containing synovial cells (P>0.05, MANCOVA).

Conclusions. The dose–response relationship of i.a. morphine analgesia is not shifted by enhanced inflammation and END expression within synovial tissue. Thus, the presence of END within inflamed synovial tissue does not seem to interfere with i.a. morphine analgesia.


Keywords: analgesia, postoperative; analgesic techniques, intra-articular; endorphins; macrophages; pain, acute; pain, postoperative; pain, mechanisms; pharmacology, morphine; receptors, opioid; surgery, orthopaedic; T lymphocytes

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Introduction

Repeated or continuous administration of centrally acting opioids is known to result in a loss of analgesic potency which becomes apparent in a rightward shift of the dose–response curve of analgesic effects.1 In humans, this has been reported for both chronic2 and acute3 opioid treatment. The loss of analgesic potency has been ascribed to changes at central opioid receptors. These central opioid receptors are also susceptible to the development of tolerance with prolonged exposure to elevated concentrations of endogenous opioids, a phenomenon that may result in cross-tolerance to exogenous opioid agonists.1 4–6

In the periphery, local analgesic effects of opioids are overt following their administration into inflamed tissue.7 8 Apart from experimental studies, this has been shown in patients with acute and chronic inflammatory pain.9 10 The analgesic

1Some of the results were presented in abstract form at the Annual Meeting of the Society of Anesthesiology (ASA), San Francisco, CA, 2000.
effect is mediated by opioid receptors on peripheral endings of sensory neurons within inflamed subcutaneous tissue. In addition, β-endorphin (END), an endogenous opioid peptide which is expressed within immune cells in inflamed tissue, can be released upon stressful stimulation and reduce pain by activation of those receptors. Under experimental conditions, this END release and subsequent analgesia is progressively augmented with increasing duration and magnitude of the inflammatory reaction. END has also been identified within inflamed synovial tissue of patients suffering from knee injury or chronic arthritis. In a previous clinical trial, we found a dose-dependency of intra-articular (i.a.) morphine analgesia. Here we examined whether enhanced inflammation and END expression within synovial tissue of patients undergoing arthroscopic knee surgery might shift the analgesic dose–response curve of i.a. morphine.

In a preliminary study using a single dose of i.a. morphine we found no diminution of its analgesic effect in the presence of synovial inflammation. This led us to hypothesize that enhanced inflammation and opioid peptide expression within the synovial tissue of surgical patients does not result in cross-tolerance, i.e. a rightward shift in the dose–response curve, to the analgesic effects of i.a. morphine.

Materials and methods

The study protocol adhered to the ethical guidelines of the International Association for the Study of Pain and was approved by the Institutional Ethics Committee of the University Hospital, Graz, Austria. Patients gave their informed written consent before taking part in this study. The study protocol followed a prospective randomized double-blinded design.

We examined 200 patients scheduled for arthroscopic knee surgery. They were aged between 19 and 70 yr, weighed between 50 and 90 kg and had American Society of Anaesthesiologists physical status I–II. Patients were excluded from the study if (i) they had a history of chronic pain or cardiovascular, respiratory, metabolic or neurological disorder, (ii) they had taken any opioids within 12 h before surgery, (iii) they had a history of drug or alcohol abuse or psychiatric disorder or (iv) they were unable to comply with the self-administration of analgesics. Patients were instructed on how to use the visual analogue scale (VAS) for pain rating and the patient-controlled analgesia (PCA) device (Graseby PC-3000).

Patients were given oral midazolam 0.1 mg kg⁻¹ 45 min before anaesthesia. Anaesthesia was induced with propofol 2 mg kg⁻¹, fentanyl 3 μg kg⁻¹ and atracurium 0.5 mg kg⁻¹ i.v., and maintained with 0.8–1.2% isoflurane and 65% nitrous oxide in oxygen.

Test solutions of different concentrations of morphine (0.01, 0.02 and 0.04%) and isotonic saline were prepared and coded by the hospital pharmacy. At the end of the procedure, 10 ml of test solution was given through the arthroscope into the patient’s knee joint. The thigh tourniquet remained in place for another 10 min and was then released. If drains were placed into the knee joint, they were clamped for the first 60 min to prevent immediate loss of the injected test solution. Using a random number table, patients were assigned to one of four i.a. treatment groups in a double-blinded manner: group I received 10 ml saline, group II received 1 mg morphine (10 ml of 0.01% solution), group III received 2 mg morphine (10 ml of 0.02% solution) and group IV received 4 mg morphine (10 ml of 0.04% solution). The morphine doses were based on a previous study which had demonstrated a dose-dependent analgesic effect within this range.

Pain intensity was evaluated using a VAS extending from 0 (no pain) to 100 (unbearable pain). VAS scores were recorded by a blinded observer at 1, 2, 3, 6, 9, 12, 18 and 24 h after the end of surgery. In addition, the time that elapsed until patients asked for the first supplemental analgesic was recorded. At the patients’ request, they received an i.v. bolus of 0.1 mg kg⁻¹ piritramide, an opioid agonist which is nearly (0.75 times) equipotent with morphine and very often used in Germany for postoperative pain therapy. Patients were then connected to a PCA device (Graseby PC-3000) which was set to deliver an i.v. bolus of 2 mg piritramide with a lockout time of 8 min. At the end of the 24 h assessment period the total consumption of piritramide was documented for each group.

The degree of sedation was assessed using a categorical five-point scale extending from 1 (awake) to 5 (asleep). In addition, patients were asked if they experienced side effects such as nausea, vomiting, pruritus or urinary retention. The were asked to grade these symptoms from 0 to 3 (0, no symptoms; 1, mild; 2, moderate; 3, severe). The duration of anaesthesia was determined as the time from induction until the first response to a verbal command. The duration of surgery was documented as the time from the first incision until the final wound closure.

During surgery two synovial biopsies of 5–10 mm² were obtained from each patient for further histological and immunohistochemical examination. The tissue was immediately fixed in formalin, dehydrated in ethanol and embedded in paraffin. Paraffin blocks were cut serially at 4 μm thickness. For characterization of inflammatory cells, serial tissue sections were stained with haematoxylin–eosin and giemsa to detect granulocytes, plasma cells and mast cells and with anti-CD3 antibody (Dako, Glostrup, Denmark; dilution 1:300) and anti-CD68 antibody (Dako, Glostrup, Denmark; dilution 1:200) to identify T lymphocytes and monocytes/macrophages, respectively. A blinded observer counted the total number of inflammatory cells per square millimetre in 15 squares per section and five sections per patient using a Zeiss microscope (objective 40×). The median number of inflammatory cells per square millimetre was calculated and patients were then categorized into two subgroups with numbers below and above the median.
The abundance of END-containing cells was examined in two or three representative samples of each category (i.e. patients with less than and with more than than the median number of inflammatory cells per square millimetre). The number of samples was limited by the amount of available synovial specimens but we have previously shown that there is a linear correlation between the number of END-containing cells and the number of inflammatory cells. According to previously published protocols, the synovial specimens were immediately fixed in 4% (w/v) paraformaldehyde with 0.2% (v/v) picric acid in 0.16 M phosphate buffer solution (pH 6.9) for 5 h, and then placed in 15% (w/v) sucrose solution at 4°C overnight. Tissues were then embedded in OCT compound (Miles Inc., Elkhart, IN). Serial tissue sections (4 μm thick) were prepared using a cryostat and were mounted onto gelatin-coated slides. Unless otherwise stated, all incubations were done at room temperature and phosphate buffered saline (PBS) was used for washing (three times for 10 min) after each step. The sections were incubated with PBS, 0.3% H2O2 and 10% methanol for 45 min to block endogenous peroxidase. To prevent non-specific binding, the sections were incubated for 60 min in PBS containing 0.3% Triton X-100, 1% bovine serum albumin (BSA), 4% goat serum and 4% horse serum (block solution). The sections were then incubated overnight at 4°C with a polyclonal rabbit END antibody (Peninsula Laboratories, Belmont, CA; dilution 1:1000) in PBS containing 1% BSA, 3% goat serum and 0.3% Triton X-100. After washing, sections were incubated for 90 min with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) (goat anti-rabbit) in PBS containing 1% BSA, 3% goat serum and 0.3% Triton X-100. After washing, sections were incubated with avidin–biotin conjugated peroxidase for 90 min. Finally, sections were stained with 3,3′-diaminobenzidine tetrahydrochloride (Sigma, Deisenhofen, Germany) containing 0.01% H2O2 in 0.05% Tris-buffered saline pH 7.6 for 3–5 min. After the enzyme reaction, the sections were washed in tapwater, dehydrated in alcohol, cleared in xylene and mounted in DPX (Merck, Darmstadt, Germany). To demonstrate specificity of staining the following controls were included: (i) preabsorption of diluted antibody against END with 5 μg ml−1 purified END (Peninsula Laboratories) for 24 h at 4°C; (ii) omission of either the primary or secondary antibody or the avidin–biotin complex.

Statistics

The primary outcome parameter was to detect significant differences in the postoperative supplemental analgesic consumption between four different i.a. treatment groups. According to a previous study, assuming an α type I error of 0.05 and a β type II error of 0.2, the minimum number of patients required per group in order to detect significant differences would be 20. Patient characteristics, sedation scores and the occurrence of side effects were analyzed by analysis of variance (ANOVA) or χ² test, respectively. Pain intensity, determined as VAS score, was analyzed by a two-factorial analysis of variance with repeated measurement design (RM-ANOVA). To test for dose-dependent effects of VAS scores, the area under the curve (AUC) for each dose of i.a. morphine was calculated using the trapezoid rule, and a subsequent linear regression ANOVA was performed. To test for differences in the total piritramide consumption and the time to first analgesic request, data were analyzed by ANOVA and post hoc by linear regression for dose-dependent effects. Following immunohistochemistry, we investigated whether the number of inflammatory cells per square millimetre contributes as a covariate to the differences in pain intensity of the four different treatment groups. To this end patients were grouped into those below versus those above the median number of inflammatory cells per square millimetre synovial tissue section. Subsequently, VAS scores (AUC), total piritramide consumption and the time to first analgesic request were displayed in relation to each patient’s individual number of inflammatory cells per square millimetre. They were then analyzed by two-factorial multivariate analysis of covariance (MANCOVA) with repeated measurement design. Probability P<0.05 was considered significant.

Results

Of the 200 patients who entered the study, eight were excluded because of open knee surgery (two in group I, four in group II and two in group IV). Fifteen patients (seven in group I, three in group II, one in group III and four in group IV) were excluded because of a lack of compliance with the study protocol (refusal of PCA pump or incomplete documentation). Seven patients (two in group I, two in group II and three in group IV) refused further participation in the study because of side effects (nausea and vomiting) which were clearly related to the postoperative consumption of piritramide. One patient in group III had to be excluded because of severe cardiac complications, and two patients in group IV because of a migraine attack and a paravascular infusion. Thus a total of 167 patients (39 in group I, 41 in group II, 48 in group III and 39 in group IV) were included in the final data analysis. There were no significant differences in age, weight, sex, duration of surgery and anaesthesia between groups (P>0.05; ANOVA and χ² test) (Table 1). None of the included patients had preoperative opioid medication at any time. The surgical procedures (Table 2) and the placement of i.a. drains (70% of patients) were distributed equally among the groups. Side effects such as nausea and vomiting were reported in 38% of patients in group I, 32% in group II, 27% in group III and 21% in group IV. Most side effects occurred more than 6 h after the end of surgery; they were not different between patients receiving i.a. saline or different doses of i.a. morphine and were never rated >1 on a four-point categorical scale. Maximum sedation scores were not different between
groups and were never >2, with the majority ≤1 (data not shown).

VAS scores in all four groups were highest, but not significantly different, at 1 h after the operation and decreased progressively until 24 h (data not shown), most likely due to the postoperative use of PCA. There were no differences in the AUC of VAS scores between the four groups (Fig. 1A). The total piritramide consumption as well as the time to the first analgesic request were significantly different (\(P<0.05\), ANOVA) between the groups. The former decreased while the latter increased linearly with increasing doses of i.a. morphine (\(P<0.05\), linear regression ANOVA) (Fig. 1B and C).

VAS scores immediately before the first supplemental analgesic request were not significantly different between groups (group I, 5.3 (SEM 0.3); group II, 4.9 (0.3); group III, 4.3 (0.3); group IV, 4.3 (0.3); \(P>0.05\), ANOVA).

The histological and immunohistochemical examination of the synovial specimens revealed a median number of 127 inflammatory cells mm\(^{-2}\). Accordingly, patients were categorized into those with low (≤127 cells mm\(^{-2}\)) and those with high (>127 cells mm\(^{-2}\)) cell numbers. The high/low ratios were 26/13 in group I, 23/18 in group II, 20/28 in group III and 16/23 in group IV. There was no significant difference in the representation of each category between the four treatment groups. There were no differences between the high/low subgroups in VAS, total piritramide consumption and time to first analgesic request (\(P>0.05\), MANCOVA), with the exception of the ‘low’ subgroup of patients with minimal inflammation receiving 4 mg i.a. morphine (Fig. 2B and C).

Consistently, the analysis of VAS (AUC), total piritramide consumption and time to first analgesic request in relation to each individual patient’s number of inflammatory cells per square millimetre revealed no influence of the latter covariate (data not shown) (\(P>0.05\), MANCOVA). The immunohistochemical experiments showed that END-containing immune cells were abundant in tissue with ‘high’ synovial inflammation but hardly detectable in tissue with ‘low’ synovial inflammation, consistent with our previous studies.13 16

**Discussion**

The major findings of this study are as follows: (i) i.a. morphine produces dose-dependent postoperative analgesia in

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**Table 1** Patient characteristics. Differences between i.a. treatment groups were not statistically different (\(P>0.05\), ANOVA and \(\chi^2\) analysis)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Age (yr)</th>
<th>Male</th>
<th>Female</th>
<th>Weight (kg)</th>
<th>Duration of anesthesia (min)</th>
<th>Duration of surgery (min)</th>
<th>Intra-operative fentanyl dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (saline)</td>
<td>39</td>
<td>37 (2)</td>
<td>22</td>
<td>17</td>
<td>73.8 (2.3)</td>
<td>69.7 (2.2)</td>
<td>40.3 (2.4)</td>
<td>0.16 (0.07)</td>
</tr>
<tr>
<td>II (1 mg morphine)</td>
<td>41</td>
<td>43 (2)</td>
<td>29</td>
<td>12</td>
<td>80.1 (1.8)</td>
<td>81.8 (5.3)</td>
<td>52.9 (4.7)</td>
<td>0.19 (0.14)</td>
</tr>
<tr>
<td>III (2 mg morphine)</td>
<td>48</td>
<td>44 (2)</td>
<td>37</td>
<td>11</td>
<td>78.5 (2)</td>
<td>80.2 (5.8)</td>
<td>51.5 (4.8)</td>
<td>0.19 (0.06)</td>
</tr>
<tr>
<td>IV (4 mg morphine)</td>
<td>39</td>
<td>45 (2)</td>
<td>26</td>
<td>13</td>
<td>79.8 (2.5)</td>
<td>74.6 (4.7)</td>
<td>46.4 (4.8)</td>
<td>0.15 (0.09)</td>
</tr>
</tbody>
</table>

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**Table 2** Number of patients and types of arthroscopic surgery

<table>
<thead>
<tr>
<th>Group</th>
<th>Meniscectomy</th>
<th>Shaving</th>
<th>Cruciate ligament repair</th>
<th>Diagnostic arthroscopy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (saline)</td>
<td>29</td>
<td>6</td>
<td>1</td>
<td>34</td>
<td>109</td>
</tr>
<tr>
<td>II (1 mg morphine)</td>
<td>24</td>
<td>12</td>
<td>1</td>
<td>4</td>
<td>37</td>
</tr>
<tr>
<td>III (2 mg morphine)</td>
<td>26</td>
<td>15</td>
<td>3</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>IV (4 mg morphine)</td>
<td>30</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>41</td>
<td>48</td>
<td>39</td>
<td>167</td>
</tr>
</tbody>
</table>
patients with both high and low degrees of synovial inflammation; (ii) END expressed within synovial inflammatory cells does not shift this dose–response relationship. The first finding is consistent with previous studies showing that i.a. morphine analgesia increases in a dose-dependent manner.17 18 Whereas in those studies dose dependency was mainly demonstrated by a decrease in pain intensity scores with increasing doses of i.a. morphine, this is now demonstrated by the linearly decreasing supplemental analgesic consumption as well as the linearly increasing time to first analgesic request with increasing doses of i.a. morphine. The decrease in supplemental analgesic intake with increasing doses of i.a. morphine apparently led to the net result that overall VAS scores displayed no differences between groups. Two recently published systematic reviews also came to the conclusion that there is evidence for a dose-dependent analgesic effect of i.a. morphine.19 20 For statistical reasons, these systematic reviews mainly considered a reduction in pain intensity, but not other outcome measures of pain relief (e.g. analgesic consumption), although all these outcomes are necessarily interrelated.21

The second and most important finding of this study is that patients with high and low synovial inflammation showed similar dose–response relationships in i.a. morphine analgesia. In particular, there was neither a right- nor leftward shift of dose–response curves, i.e. neither an increase nor a decrease in the potency of i.a. morphine. This is demonstrated by the similar dose–response curves for both supplemental opioid consumption and time to first medication request, except for the 4 mg morphine dose with respect to the latter. In addition, the individual number of each patient’s inflammatory cells had no influence on the patient’s corresponding pain scores or analgesic intake within each i.a. treatment group. Also, we found more END-containing cells in high degrees of synovial inflammation and less in low degrees. This is consistent with our experimental studies which indicate that both the higher number of END-producing cells and the content of END within inflamed tissue increase with the duration and development of inflammation.13 15 Upon stressful stimulation, END is released from these cells and acts on opioid receptors of peripheral sensory nerve endings which have also been shown within synovial tissue.12 16 22 The functional consequence of this enhanced opioid peptide expression within inflamed tissue is progressively augmented pain relief.13 15 However, this clinical trial does not show a potentiation in pain relief in the group with a high degree of synovial inflammation which may be due to the clinical circumstances that synovial inflammation is not as well controlled as under experimental conditions.

On the other side, the local expression of END within inflammatory cells of synovial tissue does not result in any alterations of the dose–response relationship of i.a. morphine analgesia. Since endogenous and exogenous opioids exert their analgesic effects through the same opioid receptors,23 the increased expression of endogenous END in marked synovial inflammation may produce cross-tolerance, i.e. a rightward shift in the analgesic dose–response curve of i.a. morphine. In fact, this has been described for central opioid analgesic actions, which appear to be exquisitely susceptible to the development of tolerance, i.e. a decrease in potency with prolonged exposure to exogenous or elevated levels of endogenous opioid agonists.14 15 In the present study, however, the enhanced presence of END-positive cells within inflamed synovial tissue did not result in the development

**Fig 2** (A) Pain intensity represented as the AUC of VAS scores (0–24 h postoperatively), (B) total piritramide consumption and (C) postoperative time to the first analgesic request in patients subgrouped into those with marked (>127 inflammatory cells mm⁻²) and minimal (<127 inflammatory cells mm⁻²) synovial inflammation. VAS scores (raw data and calculated AUC) were not significantly different (P>0.05, MANCOVA). Total piritramide consumption decreased and time to first analgesic request increased with increasing doses of i.a. morphine (P<0.05, linear regression ANOVA), except for the time to first analgesic request in patients with minimal signs of synovial inflammation (C). There were no significant differences between subgroups with marked (>127 inflammatory cells mm⁻²) and minimal (<127 inflammatory cells mm⁻²) signs of synovial inflammation, except for the time to first analgesic request in patients receiving 4 mg i.a. morphine (C). Data represent means (SEM).
of such cross-tolerance. This might be also due to changes in the functional activity of opioid receptors on peripheral sensory nerve endings which have not been investigated in this trial. While this can be done in experimental studies, it is very difficult to perform such investigations in humans.

The present results confirm a dose–response dependency of i.a. morphine analgesia. Interestingly, this can be observed in patients with both high and low degrees of synovial inflammation. Apparently, an increased number of inflammatory cells and an enhanced expression of endogenous opioids within synovial tissue do not seem to alter this dose dependency. Our findings might also be of interest for the local opioid treatment of chronic arthritic pain patients. Further studies are needed, particularly with repeated injections of i.a. morphine in such patients, to gain more insight into alterations of the effectiveness of such treatment during inflammation.

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References
6 Wu HH, Wilcox GL, McLoon SC. Implantation of AT-20 or genetically modified AT-20/hENK cells in mouse spinal cord induced antinociception and opioid tolerance. J Neurosci 1994; 14: 4806–14
20 Kalso E, Smith L, McQuay HJ, Moore RA. No pain, no gain: clinical excellence and significant rigour—lessons learned from i.a. morphine. Pain 2002; 98: 269–75