Lack of Analgesic Activity of Morphine-6-glucuronide after Short-term Intravenous Administration in Healthy Volunteers

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Background: The analgesic activity of morphine-6-glucuronide (M-6-G) is well recognized for its contribution to the effects of morphine and its possible use as an opioid analgesic with a wider therapeutic range than morphine. The present study attempted to quantify the relative contribution of M-6-G to analgesia observed after systemic administration of morphine.

Methods: In a placebo-controlled, sixfold crossover study in 20 healthy men, the effects of M-6-G were assessed at steadystate plasma concentrations of M-6-G identical to and two and three times higher than those measured after administration of morphine. Morphine and M-6-G were administered as an intravenous bolus followed by infusion over 4 h. Dosage A was M-6-G-bolus of 0.015 mg/kg plus infusion of 0.0072 mg·kg⁻¹·h⁻¹. Dosage B was M-6-G-bolus of 0.029 mg/kg plus infusion of 0.014 mg·kg⁻¹·h⁻¹. Dosage C was M-6-G-bolus of 0.044 mg/kg plus infusion of 0.022 mg·kg⁻¹·h⁻¹. Dosage D was a morphine bolus of 0.14 mg/kg plus infusion of 0.05 mg·kg⁻¹·h⁻¹ for 4 h. Dosage E was M-6-G combined with morphine (doses A + D). Dosage F was a placebo. The analgesic effects of M-6-G and morphine were measured before administration of the bolus and after 3.5 h using an experimental pain model based on pain-related cortical potentials and pain ratings after specific stimulation of the nasal nociceptor with short pulses of gaseous carbon dioxide.

Results: Morphine significantly reduced subjective and objective pain correlates compared with placebo. In contrast, M-6-G produced no statistically significant effects. The addition of M-6-G to morphine did not increase the effects of morphine. Morphine produced significantly more side effects than M-6-G.

Conclusion: After short-term intravenous administration at doses that produce plasma concentrations of M-6-G similar to those seen after administration of morphine, M-6-G had no analgesic effects in the present placebo-controlled study in healthy volunteers. (Key words: Analgesia; evoked potentials. Analgesics: morphine; morphine-6-glucuronide. Pharmacokinetics: intravenous, steady-state.)

MORPHINE-6/β-GLUCURONIDE (M-6-G) is a major metabolite of morphine with potent opioid actions. Given subcutaneously in mice, M-6-G elicited antinociception and inhibited gastrointestinal motility with an effect approximately twice that of morphine, and these actions were easily reversed by naloxone. However, when injected either intracerebroventricularly or intrathecally, M-6-G was approximately 90 times and 650 times more potent as an analgesic than morphine. Other studies have also documented that M-6-G is a potent antinociceptive agent in rats. Further, M-6-G was proposed to contribute to both analgesic effects and toxicity in morphine in humans. Particularly in patients with renal impairment, the side effects of morphine have been attributed to the increased plasma levels of M-6-G. In contrast, when renal function was not altered, M-6-G produced fewer side effects than morphine. Reports of an analgesic action of M-6-G in humans after intrathecal and intravenous administration of M-6-G have focused discussions on its possible use as a potent opioid analog.

Observations of a far higher potency of M-6-G after intracerebroventricular or intrathecal administration compared with systemic administration, however, suggested that M-6-G may distribute into the cerebrospinal fluid (CSF) to a much lesser degree than morphine. Thus the contribution of M-6-G to the effects of systemically given morphine and its qualification as
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an analgesic are still unresolved issues. The present study started from the hypothesis that systematically available M-6-G at levels similar to those seen after morphine administration contribute to the analgesic effects observed after administration of morphine. Its effects were assessed at steady-state plasma concentrations that were nearly identical and two and three times higher than the M-6-G concentrations measured after administration of morphine. To identify the relative contribution of M-6-G to analgesia observed after administration of morphine, the effects of M-6-G were compared with those of morphine and placebo. Analgesia was assessed with an established, validated experimental pain model based on both pain-related cortical potentials and pain-ratings after specific stimulation of the nasal nociceptors with short painful pulses of gaseous carbon dioxide. Concomitantly, side effects of morphine and M-6-G were assessed to allow comparison of the therapeutic indices.

Materials and Methods

Protocol
Twenty healthy men (aged 23–32 yr, mean age, 24.6 ± 2.7 yr; mean body weight, 74.2 ± 6.5 kg) participated in this randomized, double-blind, six-way crossover study (intersession interval of at least 1 week). The study was conducted according to the Declaration of Helsinki on biomedical research involving human subjects (Tokyo amendment). The protocol was approved by the University of Erlangen Ethics Review Committee. Each volunteer gave written consent for enrollment and complete procedures after detailed information was provided verbally and in writing. At the beginning and at the end of the study, the volunteers’ health was assessed by general clinical examination and routine laboratory tests with special attention to normal hepatic and renal function. Before the experiments, the volunteers abstained for 2 days from taking methylinxanthines, for 12 h from nicotine, and fasted for 6 h. Volunteers were in a supine position while the medication was administered. After completion of the infusion, the subjects received a standard meal and were free to move at will.

Dose Regimens
An intravenous bolus followed by continuous intravenous infusion (duration, 4 h) of either M-6-G or morphine were administered to reach steady-state plasma concentrations of (1) morphine after administration of morphine, (2) M-6-G after administration of morphine, and (3) M-6-G after administration of M-6-G. The doses of both morphine and M-6-G were calculated using pharmacokinetic data from a previous study. Based on other studies, we chose 35 ng/ml as a target level for morphine at steady state, with the expectation that analgesic effects would be clearly seen at that level. The resulting doses of morphine were 10 mg/70 kg for the bolus and 3.5 mg·70 kg·h⁻¹·h⁻¹ over 4 h for the infusion; these compares with clinical doses. Doses of M-6-G were chosen to produce steady-state plasma concentrations identical (dosage A), two times (dosage B), and three times higher (dosage C) than the M-6-G plasma concentrations measured after administration of morphine (dosage D). Because we previously observed a plasma concentration ratio of M-6-G: morphine of 1.5:1 after administration of morphine, the target steady-state plasma concentration of M-6-G for dosage A was defined as 52.5 ng/ml. The resulting doses of M-6-G were within the range of those reported to produce analgesic effects after intravenous administration in humans. Thus six dose regimens were chosen.

Dosage A. M-6-G: bolus of 0.015 mg/kg plus infusion of 0.0072 mg·kg⁻¹·h⁻¹ for 4 h
Dosage B. M-6-G: bolus of 0.029 mg/kg plus infusion of 0.014 mg·kg⁻¹·h⁻¹ for 4 h
Dosage C. M-6-G: bolus of 0.044 mg/kg plus infusion of 0.022 mg·kg⁻¹·h⁻¹ for 4 h
Dosage D. morphine: bolus of 0.14 mg/kg plus infusion of 0.05 mg·kg⁻¹·h⁻¹ for 4 h
Dosage E. M-6-G combined with morphine: doses A + D
Dosage F. Placebo: bolus plus infusion of saline solution (0.9% NaCl)

The combination of M-6-G and morphine was administered to cover possible pharmacokinetic or pharmacodynamic interactions between M-6-G and morphine.

Assessment of Clinical Effects
Volunteers were monitored continuously during the study. Specifically, blood oxygen saturation and heart rate were continuously recorded using a pulsoxymeter (Nellcor N-200 Pulsoxymeter, Nellcor, Haywood, CA). The presence or absence of physical and psychological effects was recorded. In addition, in concordance to previous studies with the present pain model, volunteers rated after each session the intensity of “tiredness,” “sickness,” “vertigo,” and “drowsiness” using visual analog scales (length, 100 mm) ranging from 0
(no such symptom) to 100 (symptom experienced at maximum).

**Assessment of Pain**

Pain-related parameters were assessed before (baseline) and 3.5 h after administration of the medication started. As indicators of analgesia, (1) a posttreatment decrease in pain ratings, (2) a posttreatment decrease in amplitudes of pain-related evoked potentials, and (3) a posttreatment increase of latencies of pain-related evoked potentials, relative to pretreatment values, were taken.

**Stimulation Procedures.** Experimental pain was produced by short pulses of gaseous carbon dioxide applied to the nasal mucosa. Stimuli (duration, 200 ms; stimulus rise-time, <20 ms; interstimulus interval, approximately 30 s) were applied using a device that allows for painful stimulation without concomitant alteration of mechanical or thermal conditions at the mucosa, and concentrations of the rectangular stimuli were precisely controlled. Stimulation was achieved by embedding the carbon dioxide pulses in a constantly flowing air stream (8 l/min) with controlled temperature and humidity (36.5°C, 80% relative humidity). This carrier stream was led to the right nasal cavity via a thin Teflon tube. During each session, a total of 40 stimuli of three concentrations (8 of 55% vol/vol CO₂, 16 of 60% vol/vol CO₂, and 16 of 65% vol/vol CO₂; interstimulus interval, 30 s) were applied in a pseudorandomized sequence. Only responses to the two stronger stimuli were analyzed; the lowest concentration was used to span a wide range of possible sensations that made it more difficult for the volunteers to predict the stimulus intensity. An experimental session lasted 20 min. Volunteers were seated comfortably in an air-conditioned room and acoustically shielded by white noise applied via headphones.

In an additional training session performed before commencement of the actual experiments, volunteers were acquainted with the experimental procedures. In this session, they were trained in a specific breathing technique (velopharyngeal closure) that avoids respiratory air flow inside the nasal cavity during stimulation.

**Intensity Estimates of Painful Stimuli.** Three to four seconds after presentation of each carbon dioxide stimulus, subjects estimated its intensity relative to a standard stimulus (60% vol/vol CO₂) that was given at the beginning of the first session of each experiment. Pain ratings were performed using a visual analog scale displayed on a computer monitor. The intensity of the standard was defined as 100 estimation units. For statistical evaluation, the estimates of individual volunteers were averaged separately for each carbon dioxide concentration and session.

**Chemosomatosensory Event-Related Potentials.** The electroencephalogram was recorded from five positions of the international 10/20 system (Cz, C3, C4, Fz, and Pz) referenced to linked earlobes (A1 + A2). Possible blink artifacts were monitored from an additional site (Fp2/A1 + A2). Stimulus-linked electroencephalographic segments lasting 2,048 ms were sampled with a frequency of 250 Hz (band pass, 0.2–70 Hz; prestimulus period, 512 ms). Chemosomatosensory event-related potentials were obtained by averaging the digitized electroencephalogram records (sampling frequency, 250 Hz) separately for each carbon dioxide concentration, regarding position and session. Records contaminated by eye blinks (> 40 μV in the Fp2 lead) were excluded from this process. Subsequently, base-to-peak amplitudes P1, N1, P2, their latencies, and peak-to-peak amplitude P1N1 and N1P2 of the chemosomatosensory event-related potentials were analyzed.

**Plasma Concentrations of Morphine and Its Glucuronides.** Blood samples (4 ml) were collected in potassium ethylenediaminetetraacetic acid plastic tubes before and 10, 20, 30, 40, 60 min, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, and 8 h after onset of drug administration. Plasma samples were obtained within 15 min of blood collection and were immediately stored together with quality-control samples at −25°C until analysis.

**Drug Assay.** Morphine, M-6-G, and morphine-3-glucuronide concentrations were assayed using a modified high-power liquid chromatography method as described previously. Briefly, 100 μl internal standard (hydromorphone hydrochloride, 50 μg/ml H₂O) was added to 1 ml plasma. The samples were buffered with 3 ml 0.5 M ammonium sulfate (pH, 9.3). Solid-phase extraction was performed on a system consisting of SEP-PAK VAC C18 extraction columns (1 ml, 100 mg sorbent; Millipore, Eschborn, Germany) attached to a VAC Elut vacuum manifold (Supelco, Bellefonte, PA). The extraction column is activated with 3 ml methanol, 1 ml eluant solution, and 5 ml water. After the sample is drawn through, the column is washed with 3 ml 5 mM ammonium sulfate (pH, 9.3) followed by 200 μl water. The substances are eluted from the column with 300 μl of a solution consisting of 10 mM sodium dihydrogen phosphate and acetonitrile (79:21; pH, 2.1). The
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high-power liquid chromatography system consisted of a model SP 8810 pump (Spectra Physics, Darmstadt, Germany) fitted with a model 231 autosampler (Gilson Abimed, Langenfeld, Germany), a model FP 920 fluorescent detector (Jasco, Gross-Umstadt, Germany), and a model SP 100 UV monitor (Spectra Physics, Darmstadt, Germany). The native fluorescence intensity of morphine and its metabolites were measured (excitation and emission wavelengths of 245 nm and 335 nm, respectively). The internal standard was measured at 245 nm. Separation was achieved with a prepacked C18 AB column (100 mm, 2-mm inner diameter, Macherey-Nagel, Düren, Germany). The mobile phase consisted of 10 mm sodium dihydrogen phosphate and 1.25 mm sodium dodecyl sulfate (pH adjusted to 2.2 with o-phosphoric acid) plus acetonitrile (82:18). The flow rate was 0.4 ml/min. The system was used in an air-conditioned room (20°C). The reliable limit of quantification was 10 ng/ml for all analytes (35.05 and 22.45 nm for morphine and M-6-G, respectively). The coefficients of variation over the calibration range of 10-500 ng/ml (i.e., 35.05 to 1,752.3 and 22.45 to 1,122.44 nm for morphine and M-6-G, respectively) were less than 11% for all analytes.

Medications

M-6-G with a chemical purity of more than 99.7% was supplied by Mundipharma GmbH (Limburg/Lahn, Germany). M-6-G (37.7 mg) was dissolved in Ringer’s solution (35 ml), resulting in a concentration of M-6-G of 1.05 mg/ml. This solution was sterile filtered according to the German Pharmacopoeia (DAB 10). Morphine was used as the hydrochloride commercially available (Morphin Merck 10 or 20; Merck, Darmstadt, Germany). The identity of M-6-G, especially the position of the glucuronic acid at position C-6 of the morphinan moiety, was confirmed by 1H-NMR and 13C-NMR and particularly by inversely detected heteronuclear correlation spectroscopy.

Statistical Analysis

The SPSS program (version 6.1.3 for Windows, SPSS, Chicago, IL) was used for statistical evaluation. To control for interindividual and intertreatment baseline variability, differences between data recorded after and before administration of the medication were computed (posttreatment minus pretreatment). These data were submitted to univariate analyses of variance (ANOVA) for repeated measures (within-subject factors “medication” and “stimulus intensity”). To assess differences in morphine or M-6-G to placebo, Bonferroni t tests were applied (comparisons vs. placebo), provided that the univariate ANOVAs for repeated measures yielded significant effects for the factor medication. In addition, to determine whether the additional administration of M-6-G to morphine had an effect, effects of dosages D and E were compared.

Side effects were counted and the differences in the number of side effects between the study medications were analyzed by Friedman repeated-measures ANOVA on ranks, with the Student-Newman-Keuls method as a post hoc test (pairwise comparisons of all medications). The parameters tiredness, sickness, vertigo, and drowsiness, which were estimated using a visual analog scale, were often rated 0. Because the tests for equal variance failed, these data were analyzed by Friedman repeated-measures ANOVA on ranks, with the Student-Newman-Keuls test as a post hoc test (pairwise comparisons of all medications).

Results

Clinical Effects

All volunteers completed the study. After administration of morphine, some of them showed increased activity and talkativeness lasting 2-2.5 h, followed by a period of tiredness, drowsiness, nausea, and occasional vomiting. These effects were generally mild to moderate. Naloxone (0.4-0.8 mg) and antiemetics (10 ml Vomex A [Yamanouchi Pharma GmbH, Heidelberg, Germany] given intravenously and containing 62 mg dimenhydrinate) were injected only after administration of morphine (dosages D and E) at the end of the blood sampling period to help subjects recover from nausea and drowsiness (table 1). In contrast, after administration of M-6-G, neither talkativeness nor nausea was observed. Table 1 lists the number of side effects after administration of the respective medications. Administration of morphine caused significantly more clinical effects than did M-6-G or placebo (Friedman repeated-measures ANOVA for ranks: df = 5; χ² = 71.6; P < 0.001; pairwise comparisons of all doses with the Student-Newman-Keuls method: P < 0.05 for morphine [dosages D and E] vs. placebo and P < 0.05 for morphine [dosages D and E] vs. M-6-G [dosages A, B, or C]). Significant effects of the medication were also observed for the ratings of tiredness, sickness, vertigo, and drowsiness (Friedman repeated-measures ANOVA for ranks: P < 0.001), which were rated significantly higher after administration of morphine (pairwise com-
Table 1. Number of Side Effects Observed after Administration of M-6-G, Morphine, or Placebo (n = 20)

<table>
<thead>
<tr>
<th></th>
<th>M-6-G [a]</th>
<th>Morphine [d]</th>
<th>Morphine + M-6-G [e]</th>
<th>Placebo [f]</th>
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</thead>
<tbody>
<tr>
<td>Tiredness</td>
<td>1</td>
<td>7</td>
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<td>Drowsiness</td>
<td>1</td>
<td>9</td>
<td>15</td>
<td></td>
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<tr>
<td>Dizziness</td>
<td>1</td>
<td>10</td>
<td>13</td>
<td></td>
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<tr>
<td>Vomiting</td>
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<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Vertigo</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular weakness</td>
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<tr>
<td>Hypotonia</td>
<td>1</td>
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<td></td>
<td></td>
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<tr>
<td>Orthostatic faintness</td>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
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<tr>
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<tr>
<td>Respiratory depression</td>
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<tr>
<td>Hallucinations</td>
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<td>Nightmares</td>
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<td>Hiccups</td>
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<td>Pruritus</td>
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<td>Headache</td>
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<td>Constipation</td>
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<tr>
<td>Administration of naloxone</td>
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<tr>
<td>Administration of antiemetics</td>
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<tr>
<td>Total</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>90</td>
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</table>

Dosage [a] = M-6-G bolus of 0.015 mg·kg⁻¹ plus infusion of 0.0072 mg·kg⁻¹·h⁻¹; dosage [b] = M-6-G bolus of 0.029 mg·kg⁻¹ plus infusion of 0.014 mg·kg⁻¹·h⁻¹; dosage [c] = M-6-G bolus of 0.044 mg·kg⁻¹ plus infusion of 0.022 mg·kg⁻¹·h⁻¹; dosage [d] = morphine bolus of 0.14 mg·kg⁻¹ plus infusion of 0.05 mg·kg⁻¹·h⁻¹ for 4 h; dosage [e] = M-6-G combined with morphine (doses [a] + [d]); dosage [f] = placebo.

Administration of morphine caused significantly more side effects than administration of M-6-G or placebo (Friedman repeated measures analysis of variance for ranks: df = 5; ch-square = 71.6; P < 0.001; pairwise comparisons of all dosages with the Student-Newman-Keuls method: P < 0.05 for morphine (doses [d] and [e]) versus placebo and P < 0.05 for morphine (doses [d] and [e]) versus M-6-G (doses [a], [b], or [c])).

Comparisons of all dosages with the Student-Newman-Keuls method: P < 0.05).

Pain-related Parameters

Administration of morphine significantly reduced pain ratings (by approximately 40%). In contrast, M-6-G had no significant effects of pain-related parameters compared with placebo.

Intensity Estimates of Painful Stimuli. The factor medication had significant effects on the pain ratings (ANOVA: df = 5, 18; F = 8.47; P < 0.001). Post hoc statistics revealed that the pain ratings of the two stronger stimuli (60% and 67% vol/vol CO₂) decreased significantly after administration of morphine compared with placebo (Bonferroni t tests vs. placebo: P < 0.05; fig. 2). In contrast, none of the three dosages of M-6-G produced a significant change in pain ratings. Further, the addition of M-6-G to morphine did not influence the analgesic effects of morphine (t test between dosages D and E: P < 0.05).

Chemosensory Event-related Potentials.

After administration of morphine, amplitudes of chemosensory event-related potentials decreased and latencies increased, thus meeting criteria for analgesic effects. In contrast, no changes were observed after administration of M-6-G, even at its highest dosage, C (fig. 3). A statistically significant main effect of the medication (ANOVA) could be observed for the amplitude N1P2 at recording positions Cz (df = 5, 15; F = 4.88; P < 0.001) and C4 (df = 5, 15; F = 4.64; P < 0.001), and for the latencies N1 at recording positions Cz (df = 5, 15; F = 4.39; P = 0.001) and C4 (df = 5, 15; F = 5.20;
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Fig. 1. The mean (±SD; n = 20) plasma concentration-time profiles of morphine (upper panel), M-6-G (middle), and morphine-3-glucuronide (lower panel) after intravenous administration of morphine and M-6-G. Dosage A (◊): M-6-G bolus of 0.015 mg/kg plus infusion of 0.0072 mg·kg⁻¹·h⁻¹, dosage B (○): M-6-G bolus of 0.029 mg/kg plus infusion of 0.014 mg·kg⁻¹·h⁻¹, dosage C (●): M-6-G bolus of 0.044 mg/kg plus infusion of 0.022 mg·kg⁻¹·h⁻¹, dosage D (◆): morphine-bolus of 0.14 mg/kg plus infusion of 0.05 mg·kg⁻¹·h⁻¹ for 4 h, dosage E (◇): M-6-G combined with morphine (doses A + D). After administration of M-6-G, neither morphine-3-glucuronide nor morphine was detected in plasma. Note the different scaling of the ordinates for clarification.

Fig. 2. Mean and SEM (n = 19) of the differences between pretreatment and posttreatment pain ratings (EU; Estimation units). Dosage A: M-6-G-bolus of 0.015 mg/kg plus infusion of 0.0072 mg·kg⁻¹·h⁻¹, dosage B: M-6-G-bolus of 0.029 mg/kg plus infusion of 0.014 mg·kg⁻¹·h⁻¹, dosage C: M-6-G-bolus of 0.044 mg/kg plus infusion of 0.022 mg·kg⁻¹·h⁻¹, dosage D: morphine-bolus of 0.14 mg/kg plus infusion of 0.05 mg·kg⁻¹·h⁻¹ for 4 h, dosage E: M-6-G combined with morphine (doses A + D), and dosage F: placebo. (*Bonferroni t test vs. placebo P < 0.05 after significant effect of the medication in the ANOVA.)

P < 0.001; fig. 4). Post hoc analysis identified significant differences between morphine and placebo (Bonferroni t tests vs. placebo: P < 0.05), whereas M-6-G produced no significant effects on chemosomatotopical event-related potentials.

**Plasma Concentrations of Morphine and Its Glucuronides**

Figure 1 shows plasma concentration-time curves of morphine and M-6-G. The plasma concentrations of morphine or M-6-G reached steady state approximately 1.5 h after administration of the medication was started. This was also true for M-6-G after administration of morphine. As desired, steady-state
plasma concentrations of M-6-G after administration of M-6-G were identical (dosage A), two times (dosage B) and three times higher (dosage C) than M-6-G plasma concentrations measured after administration of morphine (dosage D). After combined administration of morphine and M-6-G (dosage E), steady-state M-6-G plasma concentrations were two times higher than those seen after morphine alone. Specifically, mean steady-state plasma concentrations of M-6-G were 125.8 ± 22 nm, 254.8 ± 47 nm, 364.4 ± 48 nm, 116.6 ± 18.2 nm, and 244.6 ± 45 nm after dosages A–E, respectively, and mean steady-state plasma concentrations of morphine were 130 ± 23 nm and 141 ± 18 nm after dosages D and E, respectively. The molar plasma concentrations of M-6-G were similar (dosage A) or even higher (dosages B and C) than those of morphine. In detail, molar plasma ratios of M-6-G to morphine (dosage D) at steady state were approximately 1:1, 2:1:1, and 2:9:1 for dosages A, B, and C, respectively. Neither morphine nor morphine-3-glucuronide were detected after administration of M-6-G.

Fig. 3. Pain-related chemosomatic sensory-evoked potentials in a representative participant before (dotted lines) and after (bold lines) short-term intravenous administration of placebo, morphine, and M-6-G (recording position C2). After administration of morphine, the amplitudes decreased and the latencies increased. In contrast, after administration of M-6-G (highest dosage C), pain-related potentials did not change compared with pretreatment potentials.

Fig. 4. Mean and SEM (n = 16) of the latency N1 (top) and the amplitude N1P2 (bottom) of pain-related evoked potentials at recording position C2 ± 1 h after administration of the medication (differences to pretreatment values, 67% vol/vol CO2). After administration of morphine, amplitudes decreased and latencies increased. In contrast, after administration of M-6-G, no significant effects compared with placebo were observed.

Doseage A: M-6-G-bolus of 0.015 mg/kg plus infusion of 0.0072 mg kg⁻¹ h⁻¹, dosage B: M-6-G-bolus of 0.029 mg/kg plus infusion of 0.014 mg kg⁻¹ h⁻¹, dosage C: M-6-G-bolus of 0.044 mg/kg plus infusion of 0.022 mg kg⁻¹ h⁻¹, dosage D: morphine-bolus of 0.14 mg/kg plus infusion of 0.05 mg kg⁻¹ h⁻¹ for 1 h, dosage E: M-6-G combined with morphine (doses A + D), and dosage F: placebo. (*Bonferroni test vs. placebo P < 0.05 after significant effect of the medication in the ANOVA.)

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Table 2. Comparison between Design of the Present Study with That of Previous Studies That Reported Analgesic Effects of M-6-G after Intravenous Administration in Humans

<table>
<thead>
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<th>Study</th>
<th>Total Dose of M-6-G (mg/70 kg)</th>
<th>Study Population</th>
<th>Pain Model</th>
<th>Control</th>
<th>Statistical Evaluation</th>
<th>Analgesic Effects of M-6-G</th>
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<tr>
<td>Present study</td>
<td>iv Bolus: 1 ; iv Infusion: 2</td>
<td>20 healthy volunteers</td>
<td>CO₂ stimuli to nasal mucosa</td>
<td>Placebo and morphine</td>
<td>Analysis of variance plus Bonferroni t tests</td>
<td>No</td>
</tr>
<tr>
<td>Osborne et al.</td>
<td>1</td>
<td>20 cancer patients</td>
<td>Cancer pain</td>
<td>None</td>
<td>Multiple t tests</td>
<td>Yes</td>
</tr>
<tr>
<td>Thompson et al.</td>
<td>1.3</td>
<td>10 healthy volunteers</td>
<td>Ischemic pain</td>
<td>Morphine</td>
<td>Analysis of variance and t tests</td>
<td>Yes (significance only with t tests)</td>
</tr>
</tbody>
</table>

Discussion

M-6-G failed to produce any effects in our study. After short-term intravenous administration it caused neither clinical opioid effects nor, more importantly, analgesic effects. Significant differences between M-6-G and placebo were observed neither in subjective (pain ratings) nor in objective (evoked potentials) pain-related parameters. This contradicts the results of previous reports of an analgesic action of M-6-G after intravenous administration in humans. In contrast, morphine (i.e., the positive control) produced both clinical and analgesic effects, and the latter were seen in all pain-related parameters.

The different outcome of M-6-G in the present study compared with previous reports cannot be attributed to the dosing scheme of M-6-G used in the present study, because it was comparable to the doses for which analgesic effects have been reported (table 2). A comparative analysis of the different study designs may help to better understand the discrepancies among the studies. Previous investigations were not placebo controlled, and only Thompson et al. had a positive control (morphine). None of these reports demonstrated main effects of the different treatments by ANOVA (four different doses of M-6-G and three doses of M-6-G plus one dose of morphine, respectively). Differences between the effects of the administered dosages of M-6-G were analyzed by t tests. Because multiple t tests bear a risk of type I error, the observed effects are not statistically justified. Consequently this was recognized by Thompson et al., who interpreted their results regarding analgesic effects with great caution. Because placebo was not tested, a placebo effect also cannot be excluded. In contrast, when applying a protocol with both placebo and positive control and using ANOVA and post hoc tests with α-level adjustment, as in the present study, the effects of M-6-G could not be established.

Given the low blood-brain permeability of M-6-G compared with morphine, our results may not be surprising. Although there is no doubt that M-6-G is an opioid at the plasma concentrations usually found after injection of conventional doses of morphine, as in the present study, it may not penetrate the central nervous system in sufficient amounts to produce central clinical or analgesic effects. In contrast, in patients with compromised renal function, M-6-G may accumulate in plasma. Under those conditions of long-term high plasma concentrations, it might reach central nervous system concentrations high enough to produce central opioid effects, even with a slow and low brain uptake. Hagen et al. observed chronic nausea associated with plasma M-6-G ranging between 400 and 150 ng/ml in a patient with renal insufficiency, and Tisco et al. reported that cancer patients with plasma M-6-G levels higher than 2,000 ng/ml had either respiratory depression or obtundation. Because plasma concentrations of this magnitude were not reached in the present study, our results do not contradict the findings that side effects seen after administration of morphine may be produced by M-6-G under certain clinical conditions.

The association of high M-6-G plasma levels with opioid side effects raises questions about its therapeutic index. From its profile of receptor affinities (agonist at opioid receptors, with lower affinity to the μ₁-opioid receptor than morphine and slightly higher affinity to μ₁- and delta-opioid receptors), M-6-G may be ex-
pected to exhibit analgesic effects while eventually having fewer side effects than morphine because the analgesic actions of opioids have been related to their binding at \(\mu\), \(\kappa\), and \(\delta\)-opioid receptors,\(^{31}\) whereas it has been suggested that the \(\mu\)-opioid receptor may be responsible for many side effects.\(^{52}\) This is supported by the reports of good tolerability of intrathecal injections of M-6-G in humans.\(^{50,51}\) On the other hand, there are reports showing that M-6-G is responsible for side effects after morphine administration in patients with compromised renal function.\(^{19,19}\) This must be considered when higher intravenous doses of M-6-G will be tested in humans. The investigation of analgesic effects of higher doses of M-6-G than those used in the present report follows from the results of this study. To establish whether M-6-G qualifies as potent analgesic, higher doses of M-6-G (5, 10, or even 50 times higher) than those used in the present study should be tested. Higher doses should produce central nervous system concentrations high enough to produce central opioid effects.

The discrepancy between the results of the present study and previous suggestions of its contribution to analgesia observed with the conventional modest doses of morphine\(^{55}\) cannot be solved, stressing the low blood–brain equilibration of M-6-G. When considering reports of an intracerebral metabolism of morphine to M-6-G,\(^{74,55}\) M-6-G might be locally available after morphine has entered the central nervous system. Thus the hypothesis that M-6-G contributes to the effects of morphine\(^{53}\) does not necessarily require the ability of plasma M-6-G to cross the blood–brain barrier. It is possible that not the M-6-G in plasma but rather the M-6-G formed intracerebrally contributes to the effects of morphine.

The carbon dioxide stimuli were shown specifically to excite trigeminal nociceptors,\(^{38,39}\) and the pain ratings were demonstrated to correlate with the stimulus intensity.\(^{56}\) The use of evoked potentials as pain correlates in humans has been discussed extensively in the literature (for example see references 57–60). Further, the cortical generators of the brain potentials evoked by the carbon dioxide stimulation could be localized in the somatosensory area \(S_1\),\(^{61}\) which is assumed to be a primary projection area for nociceptive afferents.\(^{52}\) One of the advantages of this technique is that it is noninvasive, and its results seem to be poorly affected by nonanalgic drug effects.\(^{65,64}\) Analgesics established in clinical practice exhibited analgesic effects also in the present pain model (such as pentazocine, acetyl salicylic acid, tramadol, dihydrocodeine, ibuprofen, and flurbiprofen).\(^{62,65–68}\) Further, the pain model detected dose-dependent effects of analgesics\(^{67}\) and time profiles of analgesic effects.\(^{69}\) Data from direct comparisons of the present pain model with other pain models or clinical pain within one study are not available. The clinical relevance of the results obtained with the present pain model might be estimated from a comparison of the relative potencies of different analgesics, as found in the present pain model with published data.\(^{70}\) Specifically in the present study, morphine (10 mg as a bolus and 1 mg in the infusion) reduced pain-related parameters by approximately 40%. In a previous study using the present pain model, 30 mg pentazocine (intravenous bolus) reduced the chemosensory event-related potentials amplitude N1P2 by approximately 25%.\(^{32}\) Thus the relative potency of morphine to pentazocine may be estimated to be approximately 1:0.5. Fentanyl given at a dose of 0.2 mg as an intravenous bolus reduced the amplitude N1P2 by approximately 32%.\(^{56}\) These data indicate a relative potency of morphine to fentanyl of approximately 1:100. Thus the findings with the present pain model are in line with clinical experience and data published by other investigators,\(^{70}\) indicating the clinical relevance of the present results. It is unlikely that the present pain model would be selectively insensitive for M-6-G.

In conclusion, the opioid M-6-G lacks analgesic activity after short-term intravenous administration in humans at doses that produce plasma levels of M-6-G that are equipotential to those of analgesic concentrations of morphine and compare to the M-6-G plasma concentrations measured after administration of conventional doses of morphine. When applying a study design with both placebo and positive control, previously reported analgesic effects could not be reproduced. In future studies, the dose of M-6-G should be increased until analgesia will be achieved to establish its qualification as a potent opioid analgesic.

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References

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