Pharmacokinetic–Pharmacodynamic Modeling of Morphine-6-glucuronide–induced Analgesia in Healthy Volunteers

Absence of Sex Differences

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Abstract: Morphine-6-glucuronide (M6G) is a metabolite of morphine and a μ-opioid agonist. To quantify the potency and speed of onset–offset of M6G and explore putative sex dependency, the authors studied the pharmacokinetics and pharmacodynamics of M6G in volunteers using a placebo-controlled, randomized, double-blind study design.

Methods: Ten men and 10 women received 0.3 mg/kg intravenous M6G and placebo (two thirds of the dose as bolus, one third as a continuous infusion over 1 h) on separate occasions. For 7 h, pain tolerance was measured using gradually increasing transcutaneous electrical stimulation, and blood samples were obtained. A population pharmacokinetic inhibitory sigmoid Emax–pharmacodynamic analysis was used to analyze M6G-induced changes in tolerated stimulus intensity. The improvement in model fits by inclusion of covariate sex was tested for significance. P values less than 0.01 were considered significant. Taking into account previous morphine data, a predictive pharmacokinetic–pharmacodynamic model was constructed to determine the contribution of M6G to morphine analgesia.

Results: M6G concentrations did not differ between men and women. M6G caused analgesia significantly greater than that observed with placebo (P < 0.01). The M6G analgesia data were well described by the pharmacokinetic–pharmacodynamic model. The M6G effect site concentration causing a 25% increase in current (C25) was 275 ± 135 μM (population estimate ± SE), the blood effect site equilibration half-life was 6.2 ± 3.3 h, and the steepness parameter was 0.71 ± 0.18. Intersubject variability was 167% for C25, and 218% for the effect half-life. None of the model parameters showed sex dependency.

Conclusions: A cumulative dose of 0.3 mg/kg M6G, given over 1 h, produces long-term analgesia greater than that observed with placebo, with equal dynamics (potency and speed of onset–offset) in men and women. Possible causes for the great intersubject response variability, such as genetic polymorphism of the μ-opioid receptor and placebo-related phenomena, are discussed. The predictive pharmacokinetic–pharmacodynamic model was applied successfully and was used to estimate M6G analgesia after morphine in patients with normal and impaired renal function.

IN humans, morphine is metabolized to morphine-6-glucuronide (M6G), an agent that exhibits agonistic activity at the μ-opioid receptor (MOR).1–3 Because in the near future M6G will become available as an intravenous analgesic agent for postoperative pain relief, it is of interest to examine its analgesic properties in humans. Therefore, we performed a pharmacokinetic–pharmacodynamic (PK/PD) study on the influence of 0.3 mg/kg M6G (two thirds given as bolus, the remainder given as a continuous infusion over 1 h) on pain tolerance in a group of healthy men and women. This approach enabled us to obtain information on the potency and speed of onset–offset of M6G.

Animal and human studies indicate the existence of important sex differences in opioid-induced antinociception and analgesia.4–7 Recently, we observed greater morphine analgesia in women compared to men, which was related to sex differences in morphine pharmacodynamics and not to differences in its pharmacokinetics.4 Because we believe that sex dependency in opioid behavior is probably not restricted to morphine but may be an inherent property of opioid analgesics,5,6 it is of interest to assess the existence of a sex-specific dichotomy in M6G analgesia and to quantify whether such an observation is related to M6G pharmacokinetics, pharmacodynamics, or both. Furthermore, because of the metabolism of morphine to M6G, one may argue that at least part of the observed sex difference in morphine analgesia is related to M6G. Therefore, we assessed the analgesic effect of M6G in both sexes and examined the existence of sex differences in the pharmacokinetics and pharmacodynamics of M6G.

Finally, we assessed whether M6G-specific analgesia contributes to the analgesia observed after morphine infusion and whether this effect is sex dependent. To do so, we incorporated the results of our previous study on morphine and M6G and the current study on M6G in a model of morphine metabolism to M6G and a predictive PK/PD model of morphine analgesia.4

Materials and Methods

Subjects and Experimental Pain Model

Twenty volunteers (10 men and 10 women aged 19–30 yr) were recruited to participate in the protocol after approval was obtained from the Human Ethics
Committee (Commissie Medisch Ethiek, Leids Universitair Medisch Centrum, Leiden, The Netherlands: protocol No. P00.034) and after written and oral informed consent was given. The subjects were healthy and did not have a history of illicit substance abuse. All women used oral contraceptives. The subjects were asked not to eat or drink for at least 6 h before the study. Each subject participated twice in this double-blind, randomized crossover study, once receiving placebo and once receiving M6G. Sessions were at least 3 weeks apart.

After arrival in the research unit, an arterial line was placed in the left or right radial artery under local anesthesia (for blood sampling). In the contralateral, arm an intravenous line was inserted (for drug infusion). Because the subjects were naive to pain/analgesia experiments, they were trained on both sessions (by R. R. and E. S.) for approximately 1 h, during which several stimulus trains were applied. These data were discarded. After a subsequent resting period, baseline pain threshold and tolerance were assessed in triplicate. At 9:00 AM, the drug infusion was started.

Acute pain was induced by an electrical current through two surface electrodes (Red Dot; 3M, London, Ontario, Canada) placed on the skin overlaying the tibial bone (shin bone) of the left leg. The electrodes were attached to a computer-interfaced current stimulator, which was locally designed and constructed. The intensity of the noxious stimulation was increased from 0 mA in steps of 0.5 mA/1 s. The stimulus train consisted of a square-wave pulse of 0.2 ms in duration applied at 10 Hz and had a cutoff at 128 mA. The subjects were instructed to press a button on a control panel when the stimulus became painful (pain threshold) and when no further increase in stimulus intensity was acceptable (pain tolerance). After the patient pressed the pain tolerance button, the stimulus train ended. This procedure was performed three times before drug infusion and at fixed times during and after drug infusion (at times t = 5, 10, 20, 30, 40, 50, 60, 64, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, and 420 min after the start of drug infusion). The currents at which the pain threshold and tolerance occurred were automatically collected and stored on the hard disk of a computer for further analysis. The involvement of the observers (R. R. or E. S.) in the pain assessments was restricted to training the subjects and to the initiation of the stimulus train during the studies. In case blood sampling coincided with pain assessment, the pain testing preceded the sampling.

In six subjects, each 10-Hz pain test (using the computer-interfaced current stimulator) was followed by the assessment of pain indexes at 2 Hz using an electrostimulator (Innervator NS 242; Fisher & Paykel, Auckland, New Zealand) on the contralateral leg. The intensity of this noxious stimulus (pulse duration, 0.2 ms; stimulus frequency, 2 Hz) was increased at 6-s intervals in steps of 10 mA with a cutoff at 80 mA. During the stimulus train, the subjects were instructed to state “pain” when the stimulus became painful and “stop” when no further increase in stimulus intensity was acceptable. Because this procedure (and analysis) was identical to the testing performed in our previous study on morphine, it enabled us to determine whether the results of the two tests with different stimulus frequencies were comparable and hence whether the results from our current study could be compared with the results of the morphine study.

Before the current study, our 10-Hz pain model was tested in a pilot study. Initially, in six subjects, we determined the pain threshold and tolerance at 1-h intervals for 8 h, as specified above. Median coefficients of variation for pain threshold and pain tolerance were 6.3% (range, 3.8–11.8%) and 4.0% (2.2–9.0%), respectively. As judged by linear regression analysis, no systematic increase or decrease in current over time was observed for both pain indexes. Next (on another occasion), the subjects received a 30-min step increase infusion of the opioid alfentanil, after which the infusion was terminated. The infusion was steered by a computer and aimed at an increase in target plasma concentration from 0 to 50 ng/ml (for 10 min) to 100 ng/ml (10 min) and to 150 ng/ml (10 min). Pain threshold and tolerance were measured over a 5-h period. All subjects showed marked increases in pain tolerance, with little to no hysteresis between effect and estimated alfentanil plasma concentration.

Because pain tolerance is considered more reliable in detecting true opioid-induced analgesic effect, we performed the analysis (both descriptive and PK/PD modeling) on the pain tolerance data.

M6G and Placebo

The M6G was obtained from CeNeS Ltd. (Cambridge, United Kingdom) and was dissolved in normal saline. The solution contained no morphine or morphine-3-glucuronide (M3G) as tested by the local toxicology laboratory. M6G from the same batch caused powerful analgesia in mice in the hot plate and tail immersion tests. Placebo was normal saline (0.9% NaCl). The local pharmacy performed randomization and prepared the syringes on the day before the experiment. Intravenous bolus drug infusions were made over 90 s.

The dose of M6G applied in the current study (0.3 mg/kg, two thirds given as bolus over 90 s, the remainder given over 1 h) was chosen after a dose-finding study identifying which dose of M6G yields reliable analgesia greater than that observed with placebo. To that end, one of five intravenous doses of M6G, 0, 0.05, 0.1, 0.2, or 0.3 mg/kg (all given as bolus—two thirds of total dose—and continuous infusion of 1 h), were given to five separate groups of six subjects (total number of subjects participating was 30), and their an-
algesic responses, using our computer-interfaced current stimulator, were evaluated over 7 h. We observed that although M6G doses of 0.05 and 0.1 mg/kg did not produce analgesic responses greater than that observed with placebo, doses of 0.2 and 0.3 mg/kg did produce significant analgesic responses. We opted, somewhat arbitrarily, to assess the influence of 0.3 mg/kg M6G in our current study on the effect of sex on M6G analgesia.

Opioid Plasma Concentrations

At fixed times (t = 2, 5, 10, 20, 30, 40, 50, 60, 62, 65, 70, 80, 90, 105, 120, 150, 180, 240, 300, 360, and 420 min after drug bolus), 5 ml arterial blood was drawn for determination of plasma concentrations of M6G, morphine, M3G, and arterial partial pressure of carbon dioxide (Paco₂). The determination technique using solid-phase extraction and reverse-phase high-performance liquid chromatography has been published previously. For M6G, within-day coefficients of variation (6 determinations) were 2.5% at 100 nmol/l and 1.4% at 1,500 nmol/l; interday coefficients of variation (61 determinations) were 9.8% at 50 nmol/l and 10.3% at 1,700 nmol/l. Quantitation limits were set at 5 nmol/l for morphine, 60 nmol/l for M3G, and 20 nmol/l for M6G. The molecular weights of M6G and morphine are 461 and 285 Da, respectively.

Comparison of male and female M6G concentrations were made by two-tailed tests. In addition, the areas under the concentration-time curves were determined in men and women and compared using t tests. P values less than 0.05 were considered significant.

M6G versus Placebo Effect, Men versus Women

To characterize the overall effect of the tested drugs, we calculated, relative to predrug baseline, the areas under/above the effect (pain tolerance and arterial Paco₂–time curves (AUECs) using the trapezoidal rule and standardizing by the length of the study (7 h). To detect the significance of difference between placebo and M6G, a paired t test was performed on the calculated AUECs. Separate analyses were performed in men and women. To detect a sex effect, an analysis of variance was performed on the AUECs (factors: sex, subject nested within sex, treatment, sex × treatment). A significant sex difference was assumed when the factor sex or the interaction term sex × treatment was significant. P values less than 0.05 were considered significant. Values reported are mean ± SD or mean ± 95% confidence interval.

Pharmacokinetic–Pharmacodynamic Analysis of M6G Data

The pharmacokinetics and pharmacodynamics of M6G were determined sequentially with NONMEM, version V, level 1.1 (a data analysis program for nonlinear mixed-effects modeling; University of California San Francisco, San Francisco, CA), using a population approach. Two- and three-compartment models were fitted to the M6G plasma concentration–versus–time data set. The improvement of model fit by inclusion of the covariates sex, weight, and lean body mass were tested using the likelihood ratio criterion.

The population pharmacodynamic model was based on a pharmacokinetics part with individualized (Bayesian) estimates of its parameters. To eliminate the hysteresis between the estimated M6G concentrations and analgesic effect, an effect compartment was postulated. This effect compartment equilibrates with the plasma compartment with a half-life, t1/2kE0. We used the following pharmacodynamic model:

\[
\text{current}(t) = \text{baseline current} \cdot \left[1 + \left(C_e(t)/C_{25}\right)^\gamma \right]^{0.25}
\]

where \(C_e(t)\) is the effect site concentration at time t, \(C_{25}\) is the effect site M6G concentration causing a 25% increase in current for pain tolerance, and \(\gamma\) is a shape parameter. We chose to estimate \(C_{25}\) rather than \(C_{50}\) because the changes in current after M6G were relatively small compared to baseline values. Before M6G infusion, current(t) equals baseline current (\(C_e(t = 0) = 0\)).

The improvement of model fit by inclusion of the covariates sex, weight, and lean body mass were tested using the likelihood ratio criterion. The interindividual variability of each of the model parameters (baseline current, \(C_{25}\), \(t_{1/2}k_{E0}\), and \(\gamma\)) is characterized by the coefficient of variation (%CV), which is a parameter derived from the variance of the logarithm of the individual model parameters (\(\omega^2\)). P values less than 0.01 (e.g., decreases of > 6.65 in the NONMEM objective function value for one extra parameter) were considered significant.

Because the parameter distribution may well be skewed, we obtained estimates of the confidence intervals by applying the bootstrap BCa (bias-corrected and accelerated) method. To this end, 1,000 bootstrap replications of the data were subjected to the fitting procedure. Each replication consisted of 20 random selections, with replacement, from the 20 original data sets.

M6G Formation from Morphine and Contribution of M6G to Morphine Analgesia

To calculate the formation of M6G from morphine and assess the involvement of M6G in morphine analgesia, we reanalyzed the data set of our previous study, in which 20 healthy male and female volunteers received 0.13 mg/kg intravenous morphine (two thirds as bolus, the remainder over 1 h). Pain tolerance was measured, and morphine, M6G, and M3G blood samples were taken (all during the 7 h after the bolus infusion). M6G formation was modeled using a similar approach as de-
Results

The anthropometric data of the subjects are given in table 1. All subjects completed the study without major side effects. Table 2 gives a list of side effects that occurred during the M6G and placebo studies. A "heavy" feeling and respiratory depression were the most common side effects. All subjects experienced a sensation of heaviness lasting 15–20 min in the legs, arms, chest, or neck within 1 min after the infusion of M6G but not after placebo. After M6G, the increase in arterial Pco2 over time was similar in men and women (fig. 2 and table 3) and was significantly greater than the changes in arterial Pco2 after placebo (fig. 2 and table 3). Three subjects (all women) experienced mild nausea toward the end of the study; none of them vomited.

The mean increases in current over time ± 95% confidence intervals after M6G and placebo in men and women are given in figure 2. The analysis of the analgesia–time curves (AUECs) indicated that M6G produced analgesia greater than that observed with placebo in both sexes (table 3). A sex-dependent effect could not be detected. The value of AUEC/7 h indicates that M6G caused an average increase of 3.7–3.9 mA over the 7-h measurement period. Taking into account the placebo AUEC values, there was a 2–5 times greater analgesic effect after M6G than after placebo. Comparing the ratio AUEC(M6G)/AUEC(placebo) between sexes failed to show a significant difference (P = 0.1).

M6G Pharmacokinetics and Pharmacodynamics

Plasma concentrations and areas under the curve of M6G did not differ between men and women (fig. 3). The pharmacokinetic data were best described by a

Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24.1 ± 2.9 (21–29)</td>
<td>21.6 ± 2.9 (19–28)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.8 ± 9.5</td>
<td>70.7 ± 5.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>182 ± 7</td>
<td>171 ± 4*</td>
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<tr>
<td>LBM, kg</td>
<td>62.2 ± 4.5</td>
<td>50.2 ± 2.4*</td>
</tr>
</tbody>
</table>

Values are mean ± SD (range).

*P < 0.05 (t test).

LBM = lean body mass.

Table 2. Frequency of Side Effects of M6G and Placebo

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>M6G</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Subjects</td>
<td>M:F</td>
<td>No. of Subjects M:F</td>
</tr>
<tr>
<td>Dysphoria</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flushing</td>
<td>9</td>
<td>2:7</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heavy feeling</td>
<td>20</td>
<td>10:10</td>
</tr>
<tr>
<td>Itching</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>3</td>
<td>0:3</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>2</td>
<td>0:2</td>
</tr>
<tr>
<td>Respiratory depression*</td>
<td>18</td>
<td>9:9</td>
</tr>
<tr>
<td>Sedation</td>
<td>5</td>
<td>3:2</td>
</tr>
<tr>
<td>Tiredness</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urticaria</td>
<td>1</td>
<td>0:1</td>
</tr>
<tr>
<td>Vertigo/lightheadedness</td>
<td>3</td>
<td>1:3</td>
</tr>
<tr>
<td>Warm feeling</td>
<td>13</td>
<td>5:8</td>
</tr>
</tbody>
</table>

* Defined by an increase in arterial partial pressure of carbon dioxide of > 2 mmHg.

M6G = morphine-6-glucuronide.
Table 3. Area under the Effect–Time Curves for Pain Tolerance and Arterial Pco₂ in Men and Women after Placebo and M6G Administration

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>M6G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>Pain tolerance AUEC/7 h, mA</td>
<td>1.7 ± 2.4</td>
<td>3.9 ± 1.8*</td>
</tr>
<tr>
<td>Arterial Pco₂ AUEC/7 h, kPa</td>
<td>−0.2 ± 0.2</td>
<td>0.7 ± 1.1†</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>Pain tolerance AUEC/7 h, mA</td>
<td>0.7 ± 2.9</td>
<td>3.7 ± 2.4*</td>
</tr>
<tr>
<td>Arterial Pco₂ AUEC/7 h, kPa</td>
<td>0.0 ± 0.3</td>
<td>0.6 ± 0.8†</td>
</tr>
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</table>

Values are mean ± SD.

* P < 0.05 vs. placebo (paired t test).
† P < 0.01 vs. placebo (paired t test).
‡ Assessed by three-way analysis of variance.

AUEC = area under the effect–time curve; M6G = morphine-6-glucuronide; Pco₂ = partial pressure of carbon dioxide.

This is further demonstrated in figure 7, in which we plotted the increase in currents relative to baseline current (AUEC) vs. test. The temporal development of analgesia and peak effect (relative to baseline) was similar for both tests, although the variability in effect was less with the more painful stimulus. A population analysis on the pain tolerances obtained from both assays revealed that the M6G C₂₅ values were very close (differences in C₂₅ < 10%; P > 0.05, NONMEM).

M6G Formation from Morphine

Three examples of morphine and M6G pharmacokinetic data fits (best, median, and worst fits for M6G; data from Sarton et al.) are given in figure 8. It shows that our approach yielded adequate fits to the data. The fraction of morphine clearance (Fₘ) responsible for M6G formation was 0.056 ± 0.002 [10] (typical value ± SE [%CV]), and the mean transit time in the metabolic compartment was 0.44 ± 0.03 h [23]. M6G formation from morphine was independent of sex.

Contribution of M6G to Morphine Analgesia

Examples of simulations of M6G contribution to analgesia after intravenous morphine administration are given in figure 9. Figure 9A shows the 7-h effect of a single morphine bolus (0.1 mg/kg) on pure morphine and M6G analgesia in men and women with normal renal function and with renal failure (renal clearance was set to 0 ml/min). The contribution of M6G to analgesia was

three-compartment model (table 4). For none of the model parameters did inclusion of the covariates improve the model fits (P > 0.01). Neither morphine nor its 3-glucuronide was detected in the blood samples. Inspection of the individual data fits showed that the pharmacodynamic model adequately described the M6G pain tolerance data. In figure 4, examples of five data fits are given, including the best, median, and worst fits based on the coefficient of determination ($R^2$). In figure 5, the population and individual Bayesian estimates of the analoges responses are plotted. The population pharmacodynamic model parameters are given in table 4. For none of the model parameters did inclusion of the covariates (sex, weight, and lean body mass) improve the model fits. For parameters C₂₅ and $t_{1/2}k_{e0}$, we observed relatively large %CV values, indicating the large between-subject variability. Furthermore, the parameters C₂₅, $t_{1/2}k_{e0}$, and $g$ displayed a skewed distribution to the right as shown by the 50% and 95% confidence intervals obtained from the bootstrap analysis. In figure 6, the steady state M6G-concentration against analgesic effect is plotted. For comparative reasons, the data from morphine are included.

The comparison of the 2-Hz and 10-Hz tests in six subjects showed a greater current at which predrug pain tolerance was reached for the 2-Hz test (11 mA at 10 Hz vs. 40 mA at 2 Hz). This implies that the 10-Hz stimulus train is approximately 4 times more painful than the 2-Hz stimulus train. M6G caused a peak increase in current of approximately 40% of baseline in both tests, strongly indicating that the M6G analgesic effect (potency, cf. equation 1) was independent of the stimulus frequency.

Fig. 2. Ensemble averages of morphine-6-glucuronide–induced respiratory depression: mean increase in arterial partial pressure of carbon dioxide (PCO₂) (circles) ± 95% confidence intervals (CIs; broken lines) in men (A) and women (B). On average, a 0 to 5-mmHg increase in arterial PCO₂ is observed in both sexes after M6G. The thick lines represent the mean changes in arterial PCO₂ after placebo infusion.
Fig. 3. Ensemble averages of morphine-6-glucuronide (M6G) plasma concentrations ± 95% confidence intervals (CIs) in men (A) and women (B) and the respective analgesic responses ± 95% CIs (broken lines) after 0.3 mg/kg M6G (two thirds given as bolus, the remainder given over 1 h). The bottom graphs show the mean analgesic responses to placebo in men (A) and women (B).

<table>
<thead>
<tr>
<th>Table 4. Population Pharmacokinetic and Pharmacodynamic Model Parameters</th>
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<td>Pharmacokinetic parameters</td>
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<tr>
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</tr>
<tr>
<td>$V_1$, l/kg</td>
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<tr>
<td>$V_2$, l/kg</td>
</tr>
<tr>
<td>$V_3$, l/kg</td>
</tr>
<tr>
<td>$Cl_1$, l · min⁻¹ · kg⁻¹</td>
</tr>
<tr>
<td>$Cl_2$, l · min⁻¹ · kg⁻¹</td>
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<tr>
<td>$Cl_3$, l · min⁻¹ · kg⁻¹</td>
</tr>
<tr>
<td>Pharmacodynamic parameters</td>
</tr>
<tr>
<td>Baseline, mA</td>
</tr>
<tr>
<td>$C_{25}$, nM</td>
</tr>
<tr>
<td>$t_{1/2,k_{eq}}$, h</td>
</tr>
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<td>$\gamma$</td>
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</tbody>
</table>

* Confidence intervals (CIs) derived from bootstrap analysis.

$Cl_{1-3}$, clearance from the respective compartments 1–3; $C_{25}$ = effect site concentration causing a 25% increase in current; % CV = percentage coefficient of variation; $\gamma$ = shape parameter; $t_{1/2,k_{eq}}$ = blood effect site equilibration half-life; $V_{1-3}$ = three compartments for M6G.
limited in simulated subjects with normal renal function (fig. 9). As calculated from the areas under the analgesia-effect curves, $\text{AUEC}_{M6G}/(\text{AUEC}_{M6G} + \text{AUEC}_{\text{MORPHINE}})$, the M6G contribution to total analgesia ranged from 8% (women) to 15% (men). Because of the increase in effect site concentration in renal failure subjects (from approximately 10 to 50 nM), the contribution of M6G to total analgesia increased by a factor of 2 but was still relatively small: 14% and 28% in women and men with renal failure, respectively. In figure 9B, the 48-h effect of four bolus infusions (0.1 mg/kg) at 8-h intervals is shown for normal and renal failure subjects. In normal subjects, the M6G

Fig. 4. The influence of intravenous morphine-6-glucuronide on pain tolerance in five volunteers. Best, median, and worst data fits are given together with two intermediate fits. Measured (circles) and predicted (continuous line) currents at which pain tolerance is reached are shown. Goodness of fit was assessed by the coefficient of determination ($R^2$).

Fig. 5. (A) Individual Bayesian estimates and populations estimate (thick line) of the analgesic responses to morphine-6-glucuronide (M6G). (B) Individual Bayesian estimates and populations estimate (thick line) of the placebo contribution to M6G analgesia. Placebo data were analyzed with a cubic spline with two knots of the form: current(t) = $a_0 + a_1 \times t + a_2 \times t^2 + a_3 \times t^3$. A significant population placebo effect could not be demonstrated at the $P < 0.01$ level. Continuous lines = men; broken lines = women.
analgesic contribution was 6% in women and 30% in men (peak effect site M6G concentration, 50 nM). This effect increased sixfold in renal failure: 35% in women and 188% in men (peak effect site M6G concentration, 500 nM).

Discussion

We measured the analgesic effect of M6G versus placebo in healthy young men and women. Our analysis revealed that a cumulative dose of 0.3 mg/kg M6G, given over 1 h, produces analgesia greater than that observed with placebo, with equal dynamics (AUECs, potency, and speed of onset-offset) in men and women.

**Critique of Methods**

**The Acute Pain Model.** We used transcutaneous electrical stimulation to induce acute pain. During the stimulus train, the subject pressed a button to indicate that pain became intolerable without any interaction with the observers and, consequently, also without any bias of the researchers in the assessment of pain indexes. The results of the pilot study indicated that there was no habituation to the testing procedure over a 7-h period and hence that the changes in currents observed after drug infusions are related to the pharmacologic effect of the drugs tested. Furthermore, the observation that the peak analgesic effect was observed at times when the peak alfentanil effect site concentration was predicted (pilot study) is in accordance with this statement and suggests further that the analgesic effect of the opioid tested with our pain model is related to the effect site opioid concentration.

In the current study, we increased the pulse frequency (10 Hz) compared to the frequency applied in earlier studies (2 Hz). This was done to make the stimulus more painful and consequently limit the stimulus increase after drug infusion such that a cutoff value is not reached. As a result, the mean peak increase in current at 10 Hz due to M6G was only 4.5 mA (95% confidence interval, 3.3–5.7 mA; pooled data set). This contrasts the mean increase in current observed with the 2-Hz pain test during morphine infusion (> 20 mA). To determine whether the results of the two tests with different stimulus frequencies are comparable, we performed a simultaneous assessment of pain tolerance with our current (10 Hz) and previous (2 Hz) pain tests in six of our subjects during M6G infusion (the 2-Hz pain test was identical to the one we used in the previous study on morphine). Our results showed a similar temporal development of analgesia (fig. 7) and potency values for both tests. This indicates that morphine and M6G pharmacodynamic parameter values, although derived at different stimulus frequencies, are comparable and that these parameters may be used to calculate the contribution of M6G to morphine analgesia.

**Parameterization of the Pharmacokinetic–Pharmacodynamic Model.** Frequently, pharmacodynamic models incorporate C50s to describe and compare potencies. In only seven of our subjects did we observe an increase in current greater than 50% of baseline. In seven others, the increase in current was greater than 25% but less than 50% of baseline. This indicates that the effect site concentration ranges lie below C50 in 13 of the 20 volunteers and consequently that C50 values would be poorly estimated. To overcome this problem, we param-
eterized our PK/PD model to include parameter \( C_{25} \) (the M6G steady state concentration causing a 25% increase in current) which lies well within the concentration range studied for most subjects.

Sex Differences
Animal and human data indicate that sex affects \( \mu \), \( \kappa \), and \( \delta \) opioid analgesia but that the magnitude and occurrence of sex differences depend on many interacting variables, such as the drug itself, the dose, the route of administration, the pain model used, the hormonal status of the subjects, and the experimental design. Opioid-related sex differences are possibly linked to sex differences in opioid receptor binding (with higher binding in women) and/or to sex differences in recruitment of a postsynaptic analgesic pathway (G protein–coupled inward rectifying potassium channels). Previously, we observed sex differences in morphine analgesia and respiratory effect in a group of subjects similar to the ones we examined in the current study and observed

Fig. 8. Three data fits (best, median, and worst as determined by the coefficient of determination \( R^2 \) for morphine-6-glucuronide [M6G] fits) of the simultaneous analysis of morphine and M6G (data from Sarton et al.\textsuperscript{4}). For morphine, the individual pharmacokinetic parameters were fixed to the Bayesian estimates from the morphine study (Sarton et al.\textsuperscript{4}); for M6G, the typical values and their variances were fixed to their estimates from the current study, which permits individualization to the subjects of the first study. Filled circles = M6G measured; open circles = morphine measured; thin lines = morphine predicted.

Fig. 9. Simulations of the contribution of morphine-6-glucuronide (M6G) to analgesia after intravenous morphine, using population pharmacokinetic and pharmacodynamic parameters obtained from our previous\textsuperscript{4} and current studies. Simulations are performed for subjects with normal renal function and those with complete renal failure. (A and B) Morphine plasma concentration (thin continuous line) and M6G effect site concentration in men and women with normal renal function (thick continuous line) and with renal failure (broken line). (C and D) Pure morphine analgesia in men (continuous line), pure morphine analgesia in women (dotted line), and M6G analgesia in subjects with normal renal function (thick continuous line) and those with renal failure (thick broken line). The (small) changes in morphine plasma concentration due to renal failure are not shown. (A) Effect of a single bolus of morphine (0.1 mg/kg). (B) Effect of four morphine bolus infusions at 8-h intervals. The contribution of M6G to total analgesia in normal subjects is relatively small, ranging from 6 to 30% in men and women, respectively. Because of renal failure, the effect of M6G is increased by a factor of 2 after a single bolus and by a factor of 6 after the multiple infusions.
greater opioid potency in women (i.e., sex differences were related to the pharmacodynamics of morphine, not to its pharmacokinetics).4,16,17 These findings on morphine are in accordance with the findings of others and do explain the greater opioid consumption in men on morphine for postoperative pain relief.18,19 It seems surprising that M6G did not display any sex dependency with respect to its analgesic or respiratory effect (figs. 2 and 3). However, absence of sex differences may be related to the protocol (e.g., it might be due to the more painful noxious stimulation in this study compared to our previous study on morphine, and a sex difference may possibly appear at higher M6G concentrations) or may be an M6G-specific phenomenon (note that morphine and M6G act via distinct G-protein receptor complexes).20 Our findings do indicate that the previously observed sex differences in morphine analgesic or respiratory effect were unrelated to the metabolism of morphine.4,16,17

M6G Analgesia

Comparison with the Literature. Animal studies indicate long-lasting and profound antinociception after M6G injections (especially when given centrally).1–3,34 Studies in humans are more equivocal with respect to the analgesic properties of M6G.21–32 Data from clinical studies suggest that M6G contributes significantly to the analgesic effect observed after long-term morphine administration,28–30 and intrathecal M6G administration produces potent analgesia.51,52 However, some studies that focused on single intravenous bolus infusions (bolus dose ranging from 0.04 to 0.1 mg/kg) or short-term intravenous continuous infusions showed little to no analgesic effect,24,26 although others did.22,23,25,27 Our PK/PD analysis, together with the results of our dose-finding study, indicate that a bolus dose of M6G of at least 0.2 mg/kg is needed to induce analgesia greater than placebo. In this respect, our data contrasts those earlier studies that found significant analgesia at M6G doses less than 0.2 mg/kg.22,23,25,27 Factors that could explain these differences include differences in pain model and the lack of appropriate placebo controls. However, taking into account the very low M6G dose infused in these “positive” studies (all doses would result in plasma concentrations on the initial flat part of the M6G concentration–response relation; fig. 6), most of the differences in the outcome of the studies remain unexplained.

M6G Potency. In contrast to animal data,1–3,35 the analgesic potency of M6G was less when compared to morphine.4 The morphine:M6G potency ratio ranged from 1:12 (derived from \( C_{25} \)) to 1:22 (derived from \( C_{50} \)). This indicates that M6G effect site concentrations 12–22 times greater than those of morphine are needed to obtain a similar analgesic effect. In terms of dosing, a two to three times greater M6G intravenous dose was necessary for eqipotent analgesia. Our extrapolated M6G \( C_{50} \) value (700 nm; fig. 6) is in accordance with an earlier finding derived from pupil size measurements (740 nm).34

As observed by others,34,35 we experienced relatively large intersubject variability in the potency of M6G (%CV > 150%; table 4). One possible explanation is polymorphism of the \( \mu \)-opioid receptor gene.35 For example, substitution of nucleotide adenine by guanine at position 118 of the \( \mu \)-opioid receptor gene (A118G single nucleotide polymorphism or A118G SNP), resulting in the exchange from amino acid asparagine (asn) to aspartate (asp) at position 40 of the gene product, causes a reduction in M6G potency in heterozygous carriers from 714 to 1,475 \( \mu \)M (mean of all wild types, 192 \( \mu \)M; table 5). These findings indicate the importance of the A118G genetic polymorphism in part of the observed variability of M6Gs effect (i.e., potency). Our results are the first to show that humans with this specific point mutation of

### Table 5. A118G Polymorphism of the \( \mu \)-Opioid Receptor Gene and Mean Values of the Potency Parameter (\( C_{25} \))

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Subjects</th>
<th>M:F</th>
<th>( C_{25} ), ( \mu )M</th>
<th>95% CI, ( \mu )M</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>asn(^{40})asn</td>
<td>12</td>
<td>6:6</td>
<td>192</td>
<td>60–324</td>
<td>120</td>
</tr>
<tr>
<td>asp(^{40})asn</td>
<td>6</td>
<td>2:4</td>
<td>644</td>
<td>222–1,024</td>
<td>80</td>
</tr>
</tbody>
</table>

One-tailed t test of asp\(^{40}\)asn genotype against asn\(^{40}\)asn genotype: \( P = 0.01 \).

\( C_{25} \) = homozgyous for the wild-type allele of the \( \mu \)-opioid receptor gene (adenine at position 118 of the gene for both alleles—asparagine at position 40 of the gene product); asp\(^{40}\)asn genotype = heterozygous for the mutated allele of the \( \mu \)-opioid receptor gene (guanine at position 118 of the gene for one allele causing aspartate at position 40 of the gene product); \( C_{25} \) = effect site concentration causing a 25% increase in current; CI = confidence interval; %CV = coefficient of variation.
the μ-opioid receptor gene exhibit reduced analgesic responses to M6G.

Even when taking into account genetic polymorphism of the μ-opioid receptor gene, the intersubject variability in M6G analgesic responses remained relatively large (table 5). A possible explanation for at least part of the remaining intersubject variability may be related to differences in phenomena such as expectation, experience, suggestion, attention, and conditioning. These phenomena are the basis (or at least an inherent part) of placebo analgesic responses. Some information on their importance in the observed M6G response variability may possibly be obtained by simultaneously analyzing placebo and M6G responses. Such PK/PD models have been published previously. In a first approach to study whether variability in placebo responses could explain (part of) the variability in responses to M6G, we adapted our population pharmacodynamic model as follows:

\[
\text{current}(t) = [\text{baseline current}] \cdot \left[ \begin{array}{c}
\text{placebo effect}(t) \\
[\text{M6G effect}(t)]
\end{array} \right]
\]

with separate first-order random effects (\(\eta\)) on placebo and M6G responses (allowing variability in the placebo contribution to M6G analgesia) and placebo effect described by a cubic spline (a third-order polynomial of the form: \(\text{current}(t) = a_0 + a_1 \cdot t + a_2 \cdot t^2 + a_3 \cdot t^3\); fig. 5). The results of the analysis revealed that a large part of the variability in M6G effect could also be explained by the variability in the placebo component of the model (the effect of M6G was significant in the analysis \([P < 0.01]\), indicating that M6G caused greater analgesia than placebo). However, a straightforward interpretation of these results is difficult. The placebo effect (i.e., analgesic responses related to expectation, suggestion, and conditioning) is more complex than just being a (small) part of the observed M6G analgesic response as assumed in our simple model, and both opioid and nonopioid components play a role in the placebo effect. Further, it may be that the M6G and opioid-placebo components arise from distinct opioid receptor (sub)systems with differences in their kinetics and dynamics. Therefore, it is best to consider our placebo model (in contrast to the SNP analysis) as purely phenomenologic and not as mechanistic.

Taking into account all of the above, it is evident that our protocol provided important information to unearth some of the causes of the large response variability in M6G analgesic activity \((C_{25s})\). While we were able to exclude sex as cause for the observed variability, we showed the importance of the A118G genetic polymorphism of the μ-opioid receptor gene in this respect; also, other mechanisms, such as expectation, suggestion, and conditioning, do play an important role. Further clinical studies are needed to increase our insight in this matter, for example, to examine whether the magnitude of the response variability shows drug specificity.

**M6G Analgesia versus Respiratory Depression.** In common with others, we observed respiratory depression after M6G infusion (table 3 and fig. 2). Our “closed-loop” design in the current study does not permit the calculation of potency values. Therefore, we previously assessed the respiratory potency of M6G and morphine in the female subset of this study using “open-loop” conditions. Brain concentrations of 500 and 900 \(\mu\)g were needed to suppress ventilation at a fixed end-tidal \(\text{PCO}_2\) and the ventilatory response to acute isocapnic hypoxia by 25%, respectively. This indicates a potency ratio of 1:2:3 for analgesia:carbon dioxide–related ventilation:hypoxic ventilation. That is, much greater brain M6G concentrations of M6G are needed to induce significant respiratory depression than to induce analgesia. In this respect, M6G differs favorably from morphine. For morphine, the analgesic and respiratory \(C_{25}\) values were of similar magnitude (potency ratio analgesia:carbon dioxide–related ventilation:hypoxic ventilation = 1:1:1—all data from women). This important difference in morphine and M6G behavior is difficult to explain but may be related to the proposed pharmacologic differences in effector pathways involving different G-protein receptor complexes, with possibly lesser involvement of M6G specific complexes in respiratory-related brain regions or pathways. Our observations support the notion that M6G produces less respiratory effect than morphine at equianalgesic concentrations.

**Speed of Onset–Offset of M6G Analgesia**

The speed of onset–offset of M6G (i.e., the blood effect site equilibration half-life) is related to multiple factors involved in the transfer of M6G from blood to the brain and vice versa, such as cardiac output, distribution across the blood–brain barrier, diffusion of the drug to brain sites containing the appropriate μ-opioid receptors, rate-limiting factors at the receptor level, neuronal dynamics, and efflux mechanisms. With respect to the latter, M6G is a substrate of the P-glycoprotein, an ATP-dependent drug efflux pump, which is expressed in brain capillary endothelial cells. There are two previous studies exploring the effect delay of M6G. In rats, a delay \((t_{1/2\text{ke}0})\) of 1.4 h was observed (outcome parameter vocalization after electrical stimulation); in humans, a value similar to ours (6.2 h) was estimated (outcome parameter pupil constriction) with a similar variability.

It is traditionally suggested that M6G penetrates the blood–brain barrier (a medium of low polarity) much more slowly than morphine because of the more hydrophilic nature of the M6G molecule and that this is the main if not only cause for the difference in effect delay \((t_{1/2\text{ke}0})\) between morphine and M6G. However, our
population value of $t_{1/2k_{e0}}$ is at the high end of $t_{1/2k_{e0}}$ values recently observed for morphine (1.6–4.8 h), and the 95% confidence intervals clearly overlap (table 4). This may be explained by observations that under certain conditions, the morphine glucuronides possess the ability to increase their lipophilicity. This is related to the observations that (1) in media of low polarity, M6G molecules fold and mask their polar groups (increasing their lipophilicity); and (2) M6G molecules may form zwitterions (electronically neutral double ion pairs). Furthermore, there is growing evidence that the delay between M6G plasma concentration and effect (analgesia or otherwise) is only partly related to its passage through the blood-brain barrier. For example, studies in rats showed that at least 50% of the M6G and morphine antinociceptive effect delay is the result of drug distribution within the brain tissue, rate-limiting mechanisms at the receptor level, and neuronal dynamics.

The cause of the large between-subject variability in the $t_{1/2k_{e0}}$ of M6G remains obscure but may possibly be related to polymorphism in rate-limiting processes at the receptor level or variability in efficacy of the M6G efflux pump.

**Contribution of M6G to Morphine Analgesia**

Using the pharmacokinetic parameters of morphine and M6G from our previous and current studies, we estimated that the fraction of morphine resulting in M6G formation is approximately 6%, which is at the low end of values reported in the literature (6–14%). The estimation of $F_m$ and mean transit time allowed us to calculate the contribution of M6G to morphine analgesia, taking into account the pharmacodynamic data from the current study.

The estimates of M6G contribution to total analgesia after intravenous morphine infusion(s) were greater in men than women (fig. 9). Because the production of M6G from morphine is sex independent and the morphine dosing was based on body weight in both sexes, the amount of M6G produced from morphine was equivalent in men and women. However, because morphine analgesia is greater in women, the relative contribution of M6G to total analgesia was less in women than in men. The estimated M6G effect–concentration after 0.1 mg/kg morphine was small (approximately 10 nm) and resulted in little additional analgesic effect (15% and 6% of total analgesia in men and women). Even with complete absence of M6G renal clearance, the increase in M6G effect site concentration was modest (50 nm). Although its contribution to total analgesia now increases to 28% and 14% in men and women (a doubling of relative effect), it is not expected that this causes alarming toxic side effects such as respiratory depression (the $C_{25}$ for respiratory effect—ventilation at fixed carbon dioxide—is of the order of 500 nm) or sedation. With four repetitive morphine bolus infusions (0.1 mg/kg at 8 h intervals), the peak M6G effect site concentration observed was approximately 50 nm, causing a modest contribution to analgesia (6% in women and 35% in men).

Now the influence of impaired renal clearance is significant, causing peak M6G effect site concentrations of approximately 500 nm. At these M6G concentrations, its relative analgesic contribution is increased sixfold to 35% in women and 188% in men, and both respiratory depression (hypercapnic ventilation is now depressed by approximately 25%) and sedation are expected to occur. Evidently, at more frequent morphine infusions or greater morphine doses, the predicted side effects are even greater.

We are aware of the restrictions of our simulations, which are based on data from healthy volunteers. Potential shortcomings are the absence of inclusion of (1) morphine brain metabolism into M6G, (2) enterohepatic cycling of morphine and/or M6G, and (3) the possible compensation of renal failure by an increased intestinal clearance of morphine and/or M6G in our predictive model. Taking into account the available human studies on these subjects, we believe that the omission of these factors had little to no influence on the outcome of our simulations. However, these items are insufficiently studied, especially in relation to M6G. We developed our predictive PK/PD model for the following reasons: (1) to increase our insight in the behavior of morphine and M6G concentrations after intravenous morphine in healthy volunteers; (2) to predict M6G (wanted and unwanted) effects after morphine in patients with organ failure (such as occurs in renal, liver, and cerebral disease); and (3) to develop morphine dosing regimens in patients with and without organ failure. Although we succeeded in the first goal, further studies in patients are needed to decide whether our initial estimates in simulated renal failure patients may be extrapolated to actual patients or whether adaptations in model and model parameters are required.

**Appendix: Detection of the μ-Opioid Receptor Gene A118G Single-Nucleotide Polymorphism and Allele Frequency**

We initially used the Itai restriction-length polymorphism (RFLP) analysis as an easy detection method of the A118G single-nucleotide polymorphism as described by Grosh et al. Unfortunately, introduction of the G118 in the μ-opioid receptor gene (OPRM1) sequence (Genbank Acc. No. NM_000294) or inspection of sequence traces published by Loitsch et al. did not lead to the identification of an ItaI restriction site in the G118 allele. Therefore, we designed two new primers to amplify the part of exon 1 of the OPRM1 gene containing the A118G single-nucleotide polymorphism: primer OPRM1F (5'-GCTGCAACTTGTCCCACTTAGAT-3') and with a single nucleotide substitution (italicized in sequence), which creates a restriction site for the enzyme BstUI when the G118 allele is present and OPRM1R (5'-
ventilatory depression reside within the peripheral chemoreceptors.  

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