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The Transfer Half-life of Morphine-6-glucuronide from Plasma to Effect Site Assessed by Pupil Size Measurement in Healthy Volunteers

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Background: **Clinical and experimental data suggested a long delay between the plasma concentration *versus* time course of morphine-6-glucuronide and the time course of its central opioid effects. This study was aimed at the quantification of the transfer half-life ($t_{1/2,ke0}$) of this delay.**

Methods: **Pupil size was used as a measure of central opioid effect. Eight healthy volunteers (four men, four women) participated in that single-blind randomized crossover study. Median dosages administered intravenously were 0.5 mg morphine as loading dose followed by 10.7 mg given as infusion over a period of 4.7 h, and 10.2 mg M6G as loading dose followed by 39.1 mg M6G given over a period of 3.7 h. The duration of the infusion was tailored to achieve submaximum pupil constriction. The pupil diameter was assessed every 20 min for approximately 18 h. Values of $t_{1/2,ke0}$ were obtained by semiparametric pharmacokinetic-pharmacodynamic modeling.**

Results: **The estimated median $t_{1/2,ke0}$ of M6G was 6.4 h (range, 2.9–16.2 h), and that of morphine was 2.8 h (range, 1.8–4.4 h). The individual $t_{1/2,ke0}$ of M6G was always longer than that of morphine. Judged by the concentration at half-maximun effect (EC_{50}) values of the sigmoid pupil size at maximum constriction (E_{max}) model describing concentration–response relation, M6G was apparently 22 times less potent than morphine (EC_{50} = 740.5 nM [range, 500–1,520 nM] for M6G and 36.2 nM [range, 19.7–43.3 nM] for morphine). The steepness of the sigmoid E_{max} model did not significantly differ between morphine and M6G (γ = 1.9 and 2.6, respectively). To produce similar pupil effects, the M6G dose had to be 2.8 times greater than the morphine dose.**

Conclusions: **The reported numerical value of the $t_{1/2,ke0}$ of M6G in humans obtained after direct administration of M6G is a step toward a complete modeling approach to the prediction of the clinical effects of morphine. The study raises questions about the high interindividual variability of the transfer half-life between plasma and effect site (k_{e0}) values and the apparent low potency of M6G.**

THE opioid agonistic and antinociceptive actions of the morphine metabolite morphine-6-glucuronide (M6G) have been known for approximately 30 yr.[1–3] There is evidence that M6G contributes to the clinical effects of morphine. It appears to cause severe opioid side effects after morphine administration to patients with renal failure.[4–7] The opioid effects attributed to M6G develop with a remarkable delay from the rise of the M6G plasma concentrations.[4] They persist for several hours after the disappearance of high M6G plasma concentrations.[4–7] The assumption of such a long delay between the time course of the plasma concentrations of M6G and its opioid effects may also explain the lack of analgesic effects of M6G in placebo and positively controlled studies that employed short-term M6G administration at dosages that produce M6G plasma concentrations comparable to those usually found after administration of analgesic morphine doses.[8,9] In these short-term studies, M6G might not have had enough time to enter the central nervous system at sufficient amounts. The hypothesis that time is an important factor in the clinical effects of M6G is also supported by the observation that after short-term dosing intravenous morphine is 6 to 8 times more potent than oral morphine, whereas with repeated morphine administration, intravenous morphine is only 2 to 3 times more potent than oral morphine, *i.e.*, a relative rise of the potency of oral *versus* intravenous morphine.[10] *In vitro* experiments provided further support for the hypothesis that M6G reaches its site of action slowly.[11]

To date, the transfer half-life ($t_{1/2,ke0}$) of M6G between plasma and effect site has been reported from humans only at anecdotal level. In an abstract, the $t_{1/2,ke0}$ was reported to be 20 h.[12] However, this value was calculated from data obtained after administration of morphine and not after M6G itself. Similar anecdotal values of more than 36 h were calculated from sparse and noisy data of various vigilance measures from a single patient.[4] A systematic study designed to assess the $t_{1/2,ke0}$ of M6G between plasma and effect sites has not yet been published. The lack of quantitative information on the delay between the time course of the plasma concentrations and the opioid effects of M6G is the reason why a predictive pharmacokinetic-pharmacodynamic model of the clinical effects of morphine is not available to date. Such a model has to incorporate M6G as an active morphine metabolite. The present investigation was aimed at the estimation of the $t_{1/2,ke0}$ of the central nervous effects of M6G. It employed changes in pupil size over time as a measure of central opioid effects.

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Materials and Methods

Study Design

The study was conducted according to the Declaration of Helsinki on biomedical research involving human subjects (Somerset West amendment). The protocol was approved by the Johann Wolfgang Goethe–University of Frankfurt (Frankfurt, Germany) Medical Faculty Ethics Review Board. Each subject gave written consent to enrollment and complete procedures after detailed information had been provided verbally and in writing.

Eight healthy volunteers (four men and four women: age range, 24–37 yr; body weight, 54–78 kg; body height. 1.64–1.87 m) participated in this single-blind, two-way crossover study. Single-blindness was chosen for logistic reasons and was not expected to affect the major goal of calculating the pharmacokinetic–pharmacodynamic relations for the pupil effects of M6G. A placebo condition was not actually employed because day-long pupillography under nonopioid conditions in five of the eight volunteers did not show any systematic temporal changes of pupil size. Diameters randomly varied over the day with a range of 0.6–1.9 mm; the individual coefficients of variation were 1.8–6.9%. These measurements had been made at occasions other than the present study. An exception from the random variation of pupil sizes was the sixth participant, whose pupil size showed a slight linear increase over time. However, because the slope of this increase was only 0.0061 mm/h, which would result in an increase of only 0.1 mm in 16 h, it was ignored in the subsequent calculations. Therefore, like other authors,[13] we considered the lack of a placebo control as an acceptable approach for a study on pupil effects of opioids.

No alcohol or over-the-counter medication was allowed 48 hours before and during the experiments. Intake of prescription drugs or chronic medication was prohibited 14 days before and during the study except the use of oral contraceptives. Before the administration of morphine or M6G, subjects fasted for at least 6 h.

Each study day started with three baseline measurements of pupil size. Pupil size was assessed every 20 min until the baseline values were reached again after pupil diameter had been reduced by the opioid. Subjects spent most of the time lying on a bed in the same room where the experiments took place. Owing to the short interval between measurements, they did not sleep except for a few minutes, and pupil size was assessed always at least 5 min after they had awakened because they had to stand up and walk to the pupil measurement device and the device had to be placed and focused. These procedures took several minutes. Intake of food, caffeine, and alcohol-free beverages was allowed at will. Subjects were under continuous medical supervision throughout the study. All physical or psychological effects possibly related to the opioid administration were recorded. In addition, the subjects rated the intensity of tiredness, sickness, drowsiness, vertigo, euphoria, pruritus, feeling of heaviness, and hunger every hour by means of visual analog scales (100-mm scale ranging from 0 ["no such symptom"] to 100 ["symptom experienced at maximum"]). This was introduced because observations we had made previously in volunteers had suggested that these symptoms might be of interest. The duration of a study day was approximately 18 h.

Study Medication

M6G was prepared for intravenous administration in humans according to the German pharmacopoeia by Lipomed AG (Basel, Switzerland). The purity of M6G was 98.4% (Lot No. 57.1B13.1). Morphine was used as the hydrochloride commercially available (MSI® Mundipharma; Mundipharma GmbH, Limburg/Lahn, Germany).

Morphine and M6G dosing was designed to achieve submaximum rather than maximum pupil constriction. We decided to avoid maximum pupil constriction (*i.e.*, a plateau of pupil diameters between 2 and 3 mm) to enhance the calculation of the relative potencies of M6G and morphine. A *submaximum constriction* was regarded as a pupil size close to the minimum but not reaching a plateau. After preliminary experiments, we decided to use an intravenous loading dose followed by a constant-rate intravenous infusion and defined target plasma concentrations of morphine and M6G of 25 ng/ml (87.6 nM) and 1,200 ng/ml (2,589 nM), respectively. These preliminary experiments consisted of administration of increasing doses of M6G to volunteers to establish a dosing regimen able to produce the desired submaximum pupil constriction within an acceptable time. A manually controlled infusion pump system was used (Perfusor fm; Brown, Melsungen, Germany). The first dosage targeted at 500 ng/ml plasma M6G failed to produce any pupil effects within 4 h of infusion. We subsequently increased the dose for target plasma concentrations of 800, 1,000, and 1,200 ng/ml until pupils were constricted to 3 or 4 mm within 4 h. However, when applying the same infusion time to all subjects, for some of them, 4 h was needed for pupil constriction, whereas in others, pupils were already at maximum constriction with that infusion time. Therefore, we decided to vary the infusion duration on the basis of the actual individual pupil size. That is, based on preliminary experiments, the goal to achieve submaximum pupil constrictions was found to be best achieved by stopping the morphine infusion when pupils had been constricted to a diameter of 3.5 mm and by stopping the infusion of M6G at a pupil diameter of 4.5 mm. To keep the opioid effects controlled as much as possible, we preferred this kind of tailoring of the effects over a tailoring of the effects by increasing the M6G dose. Loading and infusion doses were calculated using standard equations and pharmacokinetic parameters obtained by reanalyzing a data set of morphine and M6G plasma concentrations previously published.[14] The obtained pharma-

Table 1. Pharmacokinetic Parameters of M6G and Morphine Used for Dosage Calculation

M6G	
CL_{M6G} (l/h)	8.7
V3 (l)	9.9
Q_{M6G} (l/h)	8.4
V4 (l)	5.7
Morphine	
CL_M (l/h)	95.3
V1 (l)	21.4
Q_M (l/h)	206
V2 (l)	155

Pharmacokinetic parameters of M6G and morphine used to calculate the doses to achieve predefined target plasma concentrations. Both morphine and M6G plasma concentrations are described by standard two-compartment models. CL = body clearance; Q = intercompartment clearance; V1 and V2 = volume of distribution of compartments 1 and 2, respectively. The parameters of the pharmacokinetic model were estimated by a naïve pooled NONMEM analysis of a data set that was available from a previous investigation with administration of M6G and morphine to healthy men.[13]

cokinetic parameters are listed in table 1. The loading dose was given as a short constant rate infusion (for infusion times see table 2). Fast administration of the M6G loading dose (*i.e.*, as a bolus injection) had caused unpleasantness, a general feeling of heaviness, and a sensation of constriction of the thorax. The infusion started after the administration of the loading dose was completed, and its duration was controlled by the decrease in pupil size. After the infusion had been discontinued, the pupil size continued to decrease for a period but did not reach a plateau; thus, clipping of data was avoided. The median administered doses were 0.5 mg morphine as loading dose followed by 10.7 mg given over a period of 4.7 h and 10.2 mg M6G as loading dose followed by 39.1 mg given over a period of 3.7 h. The individual doses and durations of the infusions are given in table 2.

Measurement of Pupil Diameter

Pupil size was measured using a pupillograph (CIP; Amtech GmbH, Weinheim, Germany). The device uses infrared technology with a charge coupled device–line scan camera. Measurement of pupil diameter employs the different reflection of light by iris and pupil, which enables the charge coupled device–line camera to detect the pupil margins. During measurements, the subject was sitting in front of the device with his or her chin and forehead suspended by the pupillograph to keep pupil and sensor at constant distance. The observer stood behind the device and, using a 8.9 cm video display, controlled the correct positioning of the sensors by moving them up and down or left and right until a horizontal line representing the measuring plane was placed exactly at the biggest diameter of the pupil. The resolution of the device was 0.05 mm. Each pupil diameter was the average of at least five repetitions of single measurements.

The experiments took place in a windowless room. The subjects wore sunglasses when they had to leave the laboratory, which was allowed for not more than 5 min. During intervals between pupil measurements, the light in the laboratory was kept at 13.6 lux (average of 33 single measurements in random directions throughout the room). Three minutes before pupil diameter measurements, lamps in the laboratory were turned off. The only sources of light were two computer screens; the subject's eyes were directed in a direction opposite to these screens. The light in the room had an intensity of 0.3 lux. During the measurements the subject's eyes were exposed to a light of 10.8 lux emitted by the pupillograph. Thirty seconds of adaptation to this light were found to be sufficient.

Plasma Concentrations of Morphine and M6G

Four-milliliter blood samples were collected in potassium EDTA tubes before drug administration (baseline) and at 5, 10, 20, 30, 40, and 60 min; then, every 30 min until the end of the opioid infusion; then, 5, 10, 20, 30, 40, and 60 min after the infusion was stopped; and then, every 90 min until the end of the experiment. One additional blood sample was drawn at the next day. In total, 28–31 samples were obtained per subject. Plasma was separated within 15 min of blood collection (centrifugation: 10 min at 4,000 min^{-1}) and was immediately stored with quality control samples at −25°C until analysis. Morphine, M6G, and morphine-3-glucuronide (M3G) concentrations were assayed by a liquid chromatography tandem mass spectrometry method. Linearity of the calibration curve was proven from 0.5 to 2,000 ng/ml. The coefficient of correlation for all measured sequences was at least 0.99. The intraday and interday variability was less than 10%. The lower limits of quantification were 1 ng/ml for morphine and 0.05 ng/ml for M6G and M3G.

In brief, aliquots of human plasma samples were extracted by solid phase extraction with marginal modifications as described previously.[8] The eluate was analyzed for morphine, M3G, and M6G. Specifically, 50 μl internal standard (morphine-(N-methyl-d_3)-6-β-D-glucuronide [Lipomed AG], 200 ng/ml H_2O) was added to 200 μl plasma. The samples were buffered with 500 μl ammonium sulfate, 0.5 M (pH 9.3). Solid phase extraction was performed on a system consisting of large-volume, 100-mg sorbent Chromabond C18 extraction columns (Macherey-Nagel, Düren, Germany) attached to a Visiprep vacuum manifold (Supelco, Deisenhofen, Germany). The extraction column was activated with 3 ml methanol and 5 ml H_2O. After the sample was drawn through, the column was washed with 5 ml ammonium sulfate, 5 mM (pH 9.3), followed by 200 μl H_2O. The substances were eluted from the column with 1 ml methanol. The organic solvent was evaporated to dryness by a gentle stream of nitrogen at 40°C. The residue was reconstituted with 200 μl of the mobile phase. Calibration standards were prepared in drug-free human

Table 2. Individual Dosages of Morphine and M6G and the Subjects' Demographics

		Loading dose			Infusion			Demographics			
Subject		Dose (nmol)	Duration of Administration (h)	Rate of Administration (nmol/h)	Dose (nmol)	Duration of Administration (h)	Rate of Administration (nmol/h)	Gender	Age (yr)	Weight (kg)	Height (m)
1	Morphine	1,855	0.050	37,110	38,819	4.93	7,869	M	37	68.7	180
2		1,880	0.067	28,201	24,871	3.23	7,692	M	36	70	183
3		1,865	0.050	37,293	29,917	3.60	8,310	M	28	78	187
4		1,880	0.008	225,611	43,269	5.63	7,692	M	26	72	183
5		1,880	0.013	150,407	39,903	5.19	7,692	F	24	61	167
6		1,855	0.017	111,329	38,687	4.92	7,869	F	27	65	164
7		1,880	0.020	112,805	32,695	3.57	9,167	F	29	54	165
8		1,865	0.217	8,606	36,704	4.42	8,310	F	25	63	174
Median		1,872	0.035	74,311	37,696	4.67	7,869		27.50	66.85	177.00
1	M6G	26,497	0.20	132,487	82,164	3.73	22,008				
2		26,497	0.20	132,487	61,623	2.80	22,008				
3		26,626	0.22	122,889	128,999	5.55	23,243				
4		26,497	0.20	132,487	102,705	4.67	22,008				
5		26,497	0.20	132,487	72,627	3.30	22,008				
6		26,626	0.22	122,889	86,387	3.72	23,243				
7		26,497	0.20	132,487	101,012	4.53	22,282				
8		26,497	0.20	132,487	47,318	2.15	22,008				
Median		26,497	0.200	132,487	82,164	3.73	22,008				

Individual dosages of morphine and morphine-6-glucuronide (M6G) and demographic parameters of the study participants. The slight intersubject differences in the rates of infusion were caused by the fact that different infusion pumps were used and set to one single rate but measurements of the true rates were performed after the study was completed.

plasma and assayed at the beginning of each sequence. For control of interassay variation, spiked quality control samples in plasma were measured in each run in randomized order among samples.

High-performance liquid chromatography analysis was performed using a Jasco Model No. PU-1585 pump connected to a Jasco Model No. AS-1550 autosampler (Jasco, Gross-Umstadt, Germany). Chromatographic separations were obtained under isocratic conditions using a Nucleosil C18 column (ID, 250 × 4 mm; particle size, 5 μm; pore size, 100 Å) (Macherey-Nagel, Düren, Germany). The mobile phase consisted of acetonitrile and water at a concentration of 10:90 volume/volume adjusted to pH 2.5 with formic acid. Ten microliters of the eluate was injected onto the system. The flow rate was set at 0.3 ml/min, and the run time at 20 min. M3G, M6G, and morphine eluted after 10.4, 14.3, and 16.0 min, respectively.

Mass spectrometry and tandem mass spectrometry analyses were performed on a triple quadruple mass spectrometer (API Model No. 3000®; PE Sciex, Toronto, Ontario, Canada) with a TurboIonSpray source (Perkin Elmer, Toronto, Ontario, Canada). For measurement of morphine, M3G, and M6G the positive ion mode was chosen. High-purity nitrogen was used as nebulizer, curtain, auxiliary, and collision gas. The heated turbo gas was set at 500°C. Quantitation was performed in the multiple reaction mode using nitrogen as the collision gas with collision energy of 77, 43, and 43 eV for morphine, M3G, and M6G, respectively. Precursor-to-product ion transitions of mass-to-charge ratio (m/z) 286 → 165 for morphine, m/z 462 → 286 for M3G and M6G, and m/z 465 → 289 for the internal standard morphine-(N-methyl-d_3)-6-β-D-glucuronide were used for the multiple-reaction mode with a dwell time of 400 ms. Concentrations of the calibration standards, quality controls, and plasma concentrations were evaluated by Analyst software (version 1.1; PE Sciex, Toronto, Ontario, Canada).

Although of minor importance for the present study, the prediction performance of the calculated pharmacokinetic parameters of morphine and M6G were checked. The accuracy and the bias of the predictions were calculated from plasma concentrations sampled during continuous intravenous infusions. The bias was $100 \times (C_{measured} - C_{predicted})/C_{predicted}$, and the prediction accuracy was the absolute value of this. Predicted concentrations were corrected for the true flow rates of the infusion pumps that were measured at the end of the present study (difference from the selected rates up to 10%).

Data Analysis

The relation between M6G or morphine plasma concentrations and pupil diameter was analyzed by means of semiparametric pharmacokinetic-pharmacodynamic modeling.[15] In principle, this consisted of "smoothing" the plasma concentrations (Cp) *versus* time data with linear splines. They were convoluted with the effect site concentrations (Ce), thereby estimating the rate constant of the transfer between plasma and effect site, ke0:

$$\frac{dC_e}{dt} = k_{e0} \cdot (C_p(t) - C_e). \quad (1)$$

Concentration-*versus*-effect relation was described by a sigmoid pupil size at maximum constriction (E_{max}) model. In case of morphine administration, the actual pupil size was the sum of the effects of morphine and the M6G formed from morphine:

$$\text{Pupildiameter}_{\text{Morphine}} = E_0 - \left[(E_0 - E_{max}) \cdot \frac{Ce_{Morphine}^{\gamma,Morphine}}{Ce_{Morphine}^{\gamma,Morphine} + EC_{50,Morphine}^{\gamma,Morphine}} + (E_0 - E_{max}) \cdot \frac{Ce_{M6G}^{\gamma,M6G}}{Ce_{M6G}^{\gamma,M6G} + EC_{50,M6G}^{\gamma,M6G}} \right] \quad (2)$$

where E_0 is the baseline pupil size, E_{max} the maximum constriction (*i.e.*, the smallest possible pupil diameter under opioid action), and γ is the shape parameter of the sigmoid E_{max} model. The pupil size after administration of M6G was described by

$$\text{Pupildiameter}_{M6G} = E_0 - (E_0 - E_{max}) \cdot \frac{Ce_{M6G}^{\gamma,M6G}}{Ce_{M6G}^{\gamma,M6G} + EC_{50,M6G}^{\gamma,M6G}} \quad (3)$$

The underlying assumption that the morphine found at low concentrations in plasma after administration of M6G had no effect was verified during data analysis (see Results). For E_0 and E_{max} the same values were taken for morphine and M6G. This was based on the following: (1) Baseline values did not statistically differ between morphine and M6G conditions and (2) during an ongoing study that uses much higher doses of M6G and morphine, no differences in maximum pupil constriction were observed between morphine and M6G. Relying on a recent report which concluded that M3G appears to be devoid of significant activity,[16] this metabolite was ignored in the present data analysis. Specifically, in that study, coadministration of 10 mg/70 kg body weight morphine together with 30.6 mg/70 kg body weight M3G produced no different effects from administration of 10 mg/70 kg body weight alone. When assuming that approximately 80% of a morphine dose is metabolized to M3G,[17] from the administered 35 μM morphine (corresponding to 10 mg) results 28 μM M3G. The dose of 30.6 mg M3G administered in that study was more than double that amount (*i.e.*, 66 μM). Because it had no effects at all, ignoring M3G in the present analysis does not appear to have left out an important parameter that might have influenced the effects observed after morphine administration. Data were analyzed using a two-stage approach; NONMEM (version V 1.1, NONMEM Project Group, University of California at San Francisco, San Francisco, CA) was employed for the calculations. Data after M6G administration were analyzed first. The obtained values were then introduced into the fit of the data obtained after morphine administration and kept fixed. Probably owing to our decision to avoid maximum pupil constriction, the values of E_{max} could not be reliably estimated by fit. Specifically, the estimated E_{max} values were greater than 3.5 or less than 1 mm (in some subjects, almost zero). According to our previous experiences with pupil sizes, these values were unlikely to reflect the true minimum possible pupil diameter. Therefore, we substituted the minimum pupil size from the aforementioned ongoing study that uses higher opioid doses resulting in maximum pupil constriction. Specifically, such values were available for subjects 3 through 8. For the remaining two subjects, we used the average of the values of the other subjects as minimum pupil diameter (*i.e.*, 2.7 mm).

Spontaneously reported side effects were counted, and the sums of side effects per subject were compared between morphine and M6G by means of Wilcoxon signed rank test. For the symptoms tiredness, sickness, drowsiness, vertigo, euphoria, pruritus, feeling of heaviness, and hunger that had been repeatedly rated over time, the areas under the rating *versus* time curves (AUC) were calculated separately for each side effect using the linear trapezoidal rule. The AUC values were compared between morphine and M6G by means of Wilcoxon signed rank tests separately for each symptom. The comparison of morphine and M6G with respect to side effects was repeated with the AUC values under the side effect *versus* time curves normalized to the AUC values under the pupil size *versus* time curves. Gender differences were assessed for each parameter (clinical symptoms, parameters of the pharmacokinetic-pharmacodynamic model) by means of Wilcoxon rank sum tests.

Results

Clinical Effects

All subjects completed the study. There were no side effects that would have required medical assistance or administration rescue medication. All clinical spontaneously reported symptoms and those rated on request by visual analog scales are given in table 3. When interpreting the observed side effects, one has to keep in mind that this was a single-blind study. M6G caused significantly more spontaneous reports of side effects (Wilcoxon signed rank test: $Z = -2.12$ [$P < 0.05$]), mainly more vomiting and itching, than morphine itself. The visual analog scale ratings of tiredness increased continuously over the observation period. The ratings of the other symptoms assessed by visual analog scales increased during the first hours, remained increased for several hours, and came back to zero (or close to zero [detailed data not shown]) at the end of the study day. Their time course reflected that of the opioid plasma concentrations; further analysis was not performed because of the vagueness of these data and the lack of placebo control. The AUC values for side effects did not differ between morphine and M6G. The same was true for the AUC values normalized to the pupil size *versus* time AUC values. Men rated tiredness and drowsiness after morphine significantly lower than did women (Wilcoxon

Table 3. Side Effects

Symptom	Subject No. 1	2	3	4	5	6	7	8	Statistics
Morphine									
Count									Sum
Vomiting						2	2	1	5
Hiccup	1								1
Itching	2			9	15	8	13	5	52
Thirst									0
Stuffed nose									0
Meteorism									0
Feeling of constipation									0
Difficulty urinating									0
Headache							1		1
Diarrhea								1	1
Sum	3	0	0	9	15	10	16	7	
AUC									Median
Tiredness	680	5	529	458	715	1,656	1,413	1,091	697
Sickness	1	0	8	0	37	673	249	151	22
Drowsiness	61	1	85	62	50	1,593	886	385	74
Euphoria	0	0	8	0	0	0	0	0	0
Vertigo	13	0	81	0	0	737	176	15	14
Itching	6	1	3	192	285	511	227	25	108
Feeling of heaviness	4	16	114	0	113	1,535	586	403	114
Hunger	264	4	150	322	0	41	390	84	117
M6G									
Count									Sum
Vomiting	1		1		4	1	8		15
Hiccup	1				3				4
Itching	2			16	20	6	20	21	85
Thirst			2			1			3
Stuffed nose				1					1
Meteorism				1					1
Feeling of constipation				1					1
Difficulty urinating	1								1
Headache							1		1
Diarrhea								1	1
Sum	5	0	3	19	27	8	29	22	
AUC									Median
Tiredness	326	31	818	626	536	1,168	1,101	849	722
Sickness	88	0	140	0	115	498	444	58	102
Drowsiness	80	0	443	96	180	1,051	456	74	138
Euphoria	0	0	3	0	24	6	43	0	1
Vertigo	9	0	331	24	23	368	61	1	23
Itching	14	0	2	400	232	226	649	73	150
Feeling of heaviness	67	1	349	100	155	1,244	187	227	171
Hunger	216	11	25	307	39	71	341	31	55

Clinical effects after morphine or morphine-6-glucuronide (M6G). All spontaneous reports of side effects were noted, and their total number was counted per subject (upper parts of the table for either morphine or M6G). In addition, selected side effects were regularly rated by means of visual analog scale and the areas under the side effects ratings *versus* time curves (AUC) of the side effects *versus* time curve were calculated (lower parts for either morphine or M6G). M6G caused significantly more spontaneous reports of side effects than morphine. Men rated tiredness and drowsiness significantly lower than women. Note that the study medications had been administered in a single-blind design.

rank sum test comparing the respective AUC values: Mann-Whitney U = 0; Wilcoxon W = 10; $P < 0.05$).

Plasma Concentrations of Morphine and M6G

The plasma concentrations of morphine, M6G, and M3G are given in figure 1. During infusion of morphine, median plasma concentrations of morphine were 19.8 ng/ml (69.4 nM), and during infusion of M6G, median plasma concentrations of M6G were 1,140 ng/ml (2,460 nM). Thus, the target M6G plasma concentrations were reached with a median accuracy of 9.6%. The median bias was −1.8%. The accuracy with which the target morphine concentrations were reached was comparatively lower, that is 25.3%, and the morphine dosing tended to produce slightly lower plasma concentrations than desired (bias, −15.6%). Because predictive performance of the pharmacokinetic parameters of morphine plays no role for the present study and given the small number of healthy subjects, a correction of the morphine pharmacokinetic parameters was postponed until data from a larger and more heterogeneous population will allow for population analysis. After administration of

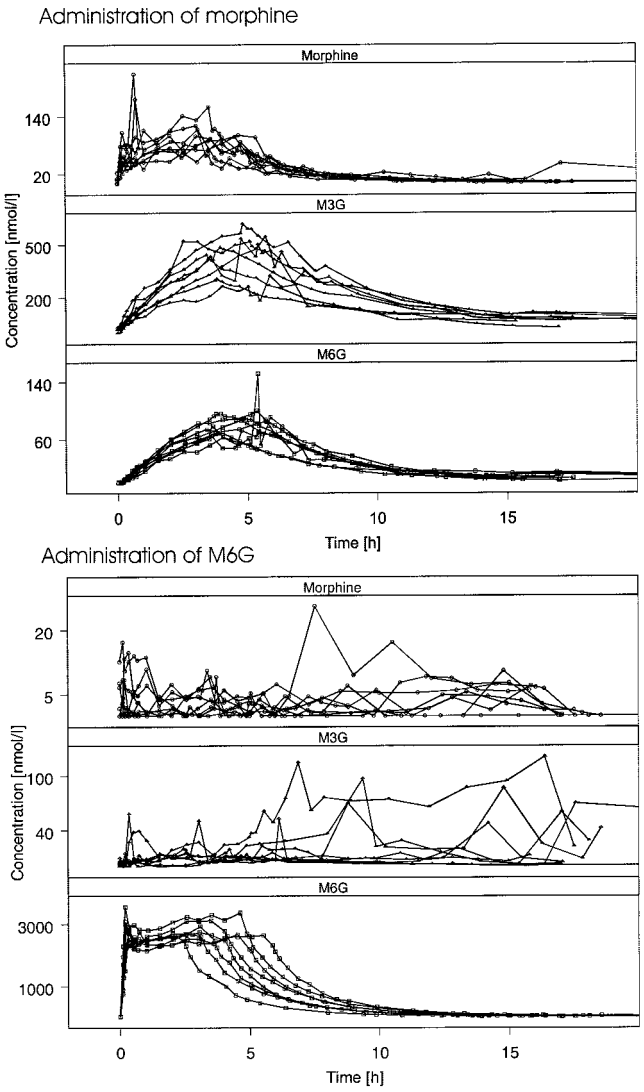

Fig. 1. Plasma concentrations of morphine, morphine-3-glucuronide (M3G), and morphine-6-glucuronide (M6G) after administration of morphine (top) or M6G (bottom) as intravenous loading dose followed by intravenous infusion at doses targeted at plasma concentrations of 25 ng/ml (87.6 nM) morphine or 1,200 ng/ml (2,589 nM) M6G, respectively. Please note the different scaling of the ordinates.

M6G, very low but measurable concentrations of morphine and M3G were found in plasma. These concentrations were close to the lower limit of quantification.

Effects of Morphine and M6G on Pupil Sizes

The time course of pupil diameters and the respective fits are given in figure 2. The numerical results of the pharmacokinetic-pharmacodynamic modeling are given in table 4. The estimated median $t_{1/2,ke0}$ of M6G was 6.4 h (range, 2.9–16.2 h), and that of morphine was 2.8 h (range, 1.8–4.4 h). The individual k_{e0} values were always smaller for M6G than for morphine (Wilcoxon signed rank test: $Z = -2.52$ [$P < 0.05$). The k_{e0} values of M6G showed a remarkable interindividual variability, with a coefficient of variation of 56.2%, contrasting with 30.7% for morphine.

The values of concentration at half-maximum effect (EC_{50}) of M6G were 22 times greater than those of morphine (Wilcoxon signed rank test: $Z = -2.52$ [$P < 0.05$). The shape parameters of the sigmoid E_{max} models did not differ statistically significantly between morphine and M6G. As shown in figure 2, the M6G did not contribute to the effects of morphine on the pupil diameter.

Considering the high EC_{50} value of M6G, the amounts of morphine found in plasma after M6G administration urged us to simulate the effects of the small amounts of morphine on pupil size to ensure that M6G, and not the morphine from deglucuronidated M6G, caused the observed pupil constriction. We repeated the NONMEM analysis for the data obtained after M6G administration but used the morphine concentrations instead of the M6G concentrations and took the pharmacodynamic parameters that we had obtained in the first analysis for morphine. These simulations were performed under the assumption that M6G had no effect at all and the changes in pupil size can be explained by the morphine plasma concentration only. The simulations clearly revealed that this was not the case. The simulated change in pupil diameter attributed to morphine found after administration of M6G resulted in almost flat lines (not shown). Thus, the pupil size after administration of M6G was only minimally influenced by the deglucuronidated morphine.

Discussion

This study quantified the delay between the time course of plasma M6G concentrations and the time course of its central opioid effects. The $t_{1/2,ke0}$ of M6G was consistently longer than that of morphine. However, the value of 6.5 h is smaller than the few anecdotal values that had been available until the present from humans (20 h, as reported in an abstract by Kramer *et al.*,[12] or greater than 36 h[4]). However, the value of 6.5 h is considerably larger than that of 1.4 h obtained in rats.[18] This points toward an interspecies difference that may be emphasized to explain why M6G is always active in animal experiments, whereas its activity after systemic administration in humans is discussed controversially. The estimated $t_{1/2,ke0}$ of morphine of 2.8 h is close to a recently published value of 3.8 h.[13]

We had published previously a simulation of the M6G and morphine effect site concentrations after repeated oral morphine administration.[19] These simulations were based on a $t_{1/2,ke0}$ of M6G of 20 h that had been taken from an abstract[12] and was, 2 yr ago, the only published value of the $t_{1/2,ke0}$ of M6G between

Table 4. PD/PD Parameters of M6G and Morphine

Subject No.	k_{e0} (h^{-1})		$t_{1/2,ke0}$ (h)		Baseline Pupil Size* (mm)		Pupil Size at Maximum Possible Constriction (mm)	EC_{50} (nmol/l)		γ	
	Morphine	M6G	Morphine	M6G	Morphine	M6G		Morphine	M6G	Morphine	M6G
1	0.29	0.09	2.4	7.5	6.9	6.5	2.7*	31.6	802	2.0	4.8
2	0.28	0.21	2.5	3.3	7.4	7.2	2.7*	39.9	1,060	3.6	1.6
3	0.16	0.13	4.4	5.4	6.0	6.5	2.5†	28.4	1,430	1.5	1.8
4	0.22	0.06	3.4	10.8	7.4	7.1	2.6†	39.5	667	1.8	3.8
5	0.24	0.13	2.9	5.2	7.5	7.1	3.0†	20.1	684	1.5	2.2
6	0.39	0.24	1.8	2.9	7.0	7.0	2.8†	37.1	1,320	1.8	2.1
7	0.26	0.04	2.7	16.2	7.0	7.1	2.3†	45.8	519	2.6	3.1
8	0.16	0.08	4.4	8.9	6.5	6.0	3.0†	31	552	2.3	4.1
Median	0.25	0.11	2.8	6.4	7.0	7.0	2.7	34.4	743	1.9	2.6
Minimum	0.16	0.04	1.8	2.9	6.0	6.0	2.3	20.1	519	1.5	1.6
Maximum	0.39	0.24	4.4	16.2	7.5	7.2	3.0	45.8	1,430	3.6	4.8

Numerical results of the semi-parametric pharmacokinetic–pharmacodynamic modeling of the effects of morphine or morphine-6-glucuronide (M6G) on pupil diameter, using a sigmoid E_{max} model. *: Baseline pupil diameter did not differ statistically significant between morphine and M6G (Wilcoxon signed rank test: $Z = -1.26$, $P = 0.208$). † Pupil diameter at maximum possible constriction after opioid administration (E_{max}; see fig. 2, top left corner) was taken from an ongoing study that uses higher doses of morphine and M6G than the present ones, achieving maximum pupil constriction (a plateau). * Pupil diameter at maximum constriction was set to the average of the values marked with †.

plasma and effect compartments from humans. At present, we updated our previous simulations by rerunning them with the new k_{e0} values. As shown in figure 3, the differences do not appear to require a correction of our past conclusions. That is, also with the new k_{e0} values, M6G can be expected to accumulate at effect site. However, considering the small EC_{50} value of M6G, we are presently reluctant to maintain our previous conclusion that these concentrations indicate a major contribution of M6G to the clinical effects after repeated morphine administration.

The fact that the EC_{50} of M6G was 22 times (range, 11–48 times) greater than the EC_{50} of morphine was not expected. From all available knowledge, M6G was considered to be more potent than morphine,[20–28] the reported relative potency ranging from a ratio of 2:1 to 650:1. Moreover, M6G was clearly shown to be more potent than morphine when injected intrathecally to patients.[27,28] In the present study, comparable effects of morphine and M6G were observed at a dose of M6G that

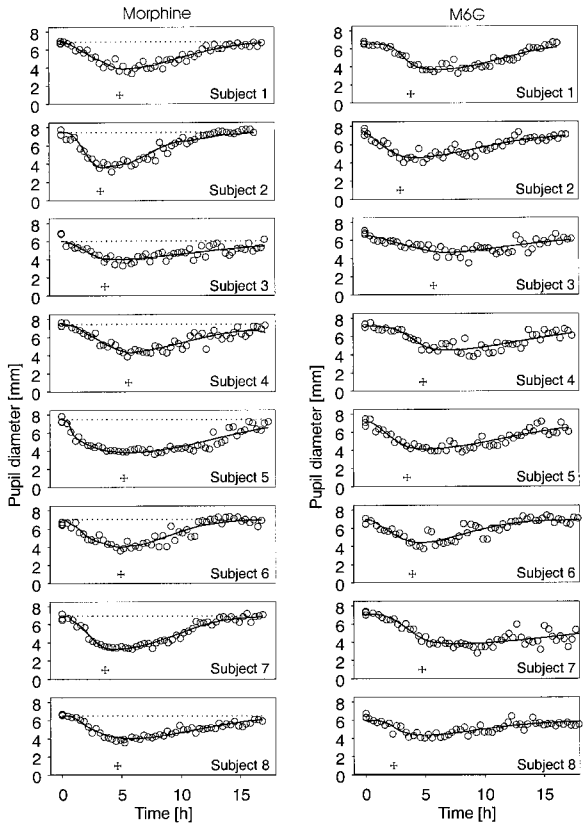

Fig. 2. Effects of morphine and morphine-6-glucuronide (M6G) administered at doses given in table 2 on pupil sizes in eight healthy volunteers. The dots depict the measured values; the solid lines the fits resulting from semiparametric pharmacokinetic–pharmacodynamic modeling (equations 1 and 2 for morphine, equations 1 and 3 for M6G). The dotted lines in the left panel show the "effects" on pupil size after administration of morphine that can be attributed to M6G. The end of opioid infusion is marked by a cross.

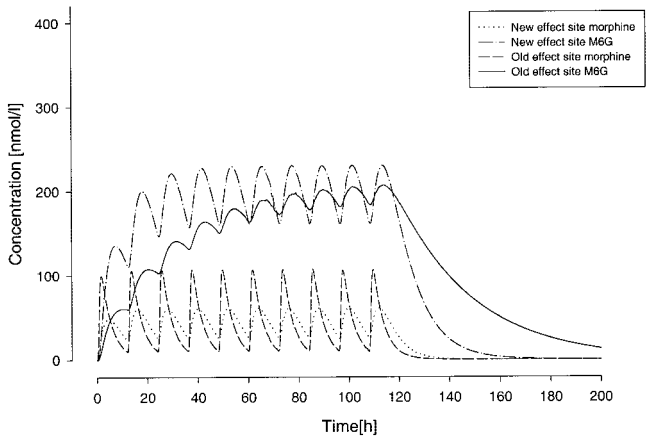

Fig. 3. Prediction of effect-compartment concentrations of both morphine and morphine-6-glucuronide (M6G) after 10 doses of 90 mg morphine (MST® Mundipharma) at 12-h interval. The simulations that were based from in our previous report[19] are called "old" in the legend. At present, simulations were rerun with the "new" time constant of the transfer half-life between plasma and effect site values obtained in the current study. The arrows point into the direction of the changes from our "old" to the "new" simulations for the effect site concentrations of M6G.

was 2.8 times greater than that of morphine. When considering that approximately 10% of morphine is metabolized to M6G,[14,29] the present amount of M6G (110,000 nM) that produced the same effects as morphine was 28 times greater than the amount of M6G (3,950 nM) formed from the morphine dose (39,500 nM) administered in the present study. This is incompatible with a view of M6G to play a major role for the effects of morphine after short-term administration.[16,30,31]

When accepting a higher potency of M6G compared with morphine, several reasons may have caused the present result of an apparently much lower potency of M6G. A first reason applies to the possibility that the pupil diameter is not a valid measure of central opioid effects. However, there is some evidence pointing against this hypothesis. Specifically, hydromorphone was found to be 10 times more potent than morphine in 13 tests including several psychophysiologic measures (tiredness, drowsiness, and so forth), respiration rate, and pupil size.[32] In another study, the relative potencies of M6G and morphine were the same for producing miosis and reducing saliva production.[33] In the latter study, M6G was reported to be four to eight times more potent than morphine at the pupils. However, that particular study did not employ administration of M6G itself but only of morphine, and the relative potency was substituted from a couple of previous studies of receptor binding. In contrast, in the present study the relative potencies of M6G and morphine were a result rather than an assumption. However, unless pupil effects and analgesia have not been assessed concomitantly within the same study, caution should be exercised when applying our pharmacokinetic–pharmacodynamic data to M6G and morphine analgesia.

The apparent 22-times-lower potency of M6G as compared with morphine may have been caused by an incomplete transfer of M6G from plasma to effect site in the sense that even at steady state M6G may not achieve similar concentrations at effect site as in plasma. This is compatible with the observation that M6G passes the blood–brain barrier not only slowly but also to a small extent.[11] The brain uptake of M6G was found to be 32-fold lower than that of morphine.[34] When dividing the EC_{50} of M6G by this value, M6G becomes more potent than morphine, and the discrepancy with the literature appears to be solved. However, that value was obtained in rats, and the extent to which results from rats on M6G brain penetration apply to humans is not clear. A low M6G brain uptake is also compatible with the very high doses of M6G of up to 125 mg within 6 h that had to be administered to achieve analgesia in women after hysterectomy.[35] High doses of M6G appear to be needed to achieve clinical effects in humans. In the present study, the total molar dose of M6G was 2.8 times the dose of morphine and the effects on pupil size were the same.

Another reason for the apparent lower potency of M6G as compared with morphine may be an uncommonly intensive development of short-term tolerance to M6G. Because the present study was not designed to assess drug tolerance, we cannot exclude this possibility. However, this issue is worth addressing in future studies.

After intravenous administration of M6G, low concentrations of morphine were measured in plasma. We calculated the areas under these morphine plasma concentrations *versus* time curves. By comparing these areas with the AUC values for morphine found after administration of morphine, it became evident that the amounts of morphine found after M6G administration were greater than the impurity of the administered M6G would have allowed for. This points toward a deglucuronidation of M6G that contrasts with our previous publications.[12,36] This apparent discrepancy may be easily explained by (1) the comparatively much higher doses of M6G producing up to 10 times higher M6G plasma concentrations than found in those previous studies and (2) the 20-times-lower limit of quantification of the present liquid chromatography–tandem mass spectrometry analysis. Because the amounts of morphine were only small, the assumption that enterohepatic cycling plays only a minor role in the pharmacokinetics of M6G[37] is not challenged by our present data.

At comparable effects on pupil size, M6G caused more side effects than did morphine. This is in line with the view of M6G as a major determinant for opioid intoxication after morphine treatment in patients with renal failure.[4] However, it contrasts with reports of a better tolerability of M6G as compared with morphine.[35,38] More information on comparative M6G toxicity has to be accumulated to finally judge this issue.

In summary, the present study for the first time assessed systematically the delay between the time course of plasma M6G concentrations and the time course of its central opioid effects. This is a step toward a modeling approach to the clinical pharmacology of morphine and facilitates the development of a predictive model of the clinical effects of morphine. The study also raised several questions, the two most important issues being the source of the high interindividual variability of the k_{e0} values and the unexpected apparent low potency of M6G as compared with morphine.

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