Pharmacokinetic modeling to predict morphine and morphine-6-glucuronide plasma concentrations in healthy young volunteers

Objective: **This investigation focused on the development of a predictive model of morphine, including morphine-6-glucuronide (M6G) for healthy young volunteers after morphine administration.**
Methods: **Population compartmental pharmacokinetic modeling with NONMEM was applied to the plasma concentration–time data of morphine and M6G obtained from 8 healthy volunteers (4 men and 4 women; age range, 23 to 30 years) after intravenous bolus injection of 5.64 mg morphine base (7.5 mg morphine sulfate) and of 1 mg deuterium-labeled M6G.**
Results: **Two models were identified that described the plasma concentration versus time courses of morphine and M6G after administration of morphine. The model consisted of a standard 3-compartment model for morphine and a standard 2-compartment model for M6G, with input into and output from the central compartments. The formation of M6G from morphine was modeled as a fraction of morphine clearance of about 14%, which accounted for the formation of M6G, and a delay of the appearance of M6G in plasma modeled as a first-order process, with a mean metabolic transit time of 17.2 minutes. An alternative model assigned the formation of M6G among the first peripheral compartment of morphine and the central compartment of M6G. Therefore the alternative 3-compartment model of morphine had the input into the central compartment and renal excretory elimination from the central compartment, but the metabolic clearance of morphine started from the first peripheral compartment. M6G was again modeled with a standard 2-compartment model. Both models predicted morphine and M6G plasma concentrations available from an independent study with acceptable accuracy and without bias.**
Conclusions: **Two models are provided that can predict plasma concentrations of morphine and M6G with acceptable accuracy in healthy young volunteers. (Clin Pharmacol Ther 2002;72:151-62.)**

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Morphine is the most frequently administered opioid analgesic agent, and morphine formulations continue to appear on the market. Launching is frequently preceded by some assessment of comparative efficacy. Morphine is also a standard substance for many investigations in pain research. These studies are often performed with healthy young volunteers, which is not the optimum selection of a study population because it excludes interindividual differences of potential clinical importance,[1] such as age or ethnic origin. Nevertheless, subject availability and the time line of the studies often result in this limited selection of study participants.

When planning studies with morphine, pharmacokinetic parameters may be needed to establish the optimum dosing regimen for the actual study goal. There are many published pharmacokinetic analyses for morphine, but only a few include the active[2,3] metabolite morphine-6-glucuronide (M6G) as an integral part of

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Table I. Demographics of the study participants

Subject No.	*Sex*	*Age (y)*	*Weight (kg)*	*Height (cm)*	*BSA (m^2)*
1	Male	29	78	187	2.03
2*	Male	25	74	183	1.95
3	Male	25	76	180	1.95
4	Male	26	98	185	2.22
5	Female	30	54	165	1.59
6	Female	28	65	164	1.71
7	Female	23	58	170	1.67
8	Female	25	61	167	1.69
Average	—	26.4	70.5	175.1	1.85

BSA, Body surface area according to Dubois.[8]

*The D_3-morphine-6-glucuronide data from this subject were excluded from the pharmacokinetic analyses because of a highly probable dosing error with the D_3-morphine-6-glucuronide.

the pharmacokinetic model.[4] Our investigation analyzed the pharmacokinetics of morphine and M6G in healthy young volunteers, with a special focus on the development of a predictive model of morphine that included M6G. In a prospective study, morphine and D_3-labeled M6G were administered. Deuterium-labeled M6G (D_3-M6G) was given to assess the disposition of M6G. The pharmacokinetic parameters of morphine and M6G were subsequently used to predict the plasma concentrations available from 8 other healthy young volunteers from a study that was completely independent of this one.

METHODS

Volunteers, study design, and study medication

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

Eight healthy volunteers (Table I) participated in this open single-occasion study. Their health was checked by physical examinations and routine laboratory tests, which showed normal values (according to the laboratory's standard) for all participants. After the subjects fasted overnight, they received three fourths of a standard 10-mg vial of morphine sulfate (MSI 10, Mundipharma GmbH, Limburg/Lahn, Germany), which corresponded to 5.64 mg morphine base as an intravenous bolus injection. Together with the morphine, 1 mg D_3-M6G was injected. The D_3-M6G was prepared for intravenous administration in humans according to the *German Pharmacopoeia* by Lipomed AG (Basel, Switzerland; purity of 98.21%; lot No. 48.1B3.1). Blood was sampled for 9 hours, and the subjects were received continuous medical supervision during that time. After a check for almost complete disappearance of any opioid side effects, the subjects were dismissed at the end of the study day.

Plasma concentrations of morphine, M6G, and D_3-M6G

Blood samples (4 mL) were collected in potassium ethylenediaminetetraacetic acid tubes before drug administration (baseline), at 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 minutes, and then every hour until the end of the study day (approximately 9 hours from the injection). The true sampling times were noted exactly and were used in the subsequent pharmacokinetic calculations. Plasma was separated within 15 minutes of blood collection and was stored with quality control samples at −25°C until analysis. Morphine, M6G, and morphine-3-glucuronide (M3G) concentrations were assayed by use of liquid chromatography–tandem mass spectrometry (LC-MS-MS) as described previously,[5] with the modification that 6-fold deuterium-labeled M6G (courtesy of Dr U. Hoffmann, Margarete Fischer-Bosch Institute for Clinical Pharmacology, Stuttgart, Germany) was used as an internal standard for both M6G and D_3-M6G. The lower limit of quantification was 1 ng/mL for morphine (3.5 nmol/L) and 0.5 ng/mL for M6G, D_3-M6G, and M3G (1.08 nmol/L). The coefficient of variation over the calibration range of 0.5 (or 1) to 1250 ng/mL was less than 5%.

Pharmacokinetic analysis

Data were analyzed by use of the nonlinear mixed-effects modeling approach of NONMEM V (release 1.1, NONMEM Project Group, UCSF, San Francisco, Calif).[6] This estimated the structural parameters of the

pharmacokinetic models together with their interindividual variance in one single step. The interindividual variability of the pharmacokinetic parameters was assumed to be log-normally distributed. A parameter value of an individual (i) is therefore given by the following:

$$P_i = P_{TV} \cdot e^{\eta i}$$

in which P_{TV} is the typical value of the respective parameter in the population, and η is a parameter with a mean of zero and a variance of ω^2 (for details, see Boeckmann et al[6]). Structural model parameters and their interindividual variances were introduced into the model in a stepwise fashion. Whether or not a specific variable remained part of the final model was decided on the basis of the likelihood ratio test. The number of degrees of freedom was equal to the difference in the number of free parameters between full and reduced models (α level of .05). If introduction of a parameter into the model resulted in a significant decrease of the NONMEM objective function, then it was indicated that the fit was improved by the respective parameter and it therefore remained part of the model, provided that the 95% confidence interval of its estimate did not include zero. Goodness of fit was also assessed by visual inspection of the fits together with observed data and by examination of the median absolute weighted residuals, calculated as follows:

$$|\text{Measured} - \text{Predicted}|/\text{Predicted}$$

on the basis of population predictions, and the median weighted residuals, calculated as follows:

$$(\text{Measured} - \text{Predicted})/\text{Predicted}$$

also on the basis of population predictions.[7]

Linear and exponential associations of subjective covariates (weight, height, age, sex, lean body mass, body surface area [BSA],[8] creatinine clearance,[9] liver enzymes, and plasma albumin) to the structural parameters of the model were analyzed analogously. The measured plasma concentrations differed from the concentrations predicted by the model by the following:

$$\log(C_{observed}) = \log(C_{predicted}) + \epsilon$$

in which ϵ is a parameter with a mean of zero and a variance of σ^2 that describes a proportional error in the logarithmic domain. We converted estimates of variance components (ω^2 and σ^2) from NONMEM into percent coefficients of variation of the parameter in the population by taking their square root and multiplying them by 100. NONMEM calculations were performed with logarithmically transformed data by use of the first-order estimation method. All data were fitted in one single step.

Model A (Fig 1, *A*) described the disposition of both morphine and M6G by use of standard compartmental models, with input into and output from the central compartments. The disposition of morphine was best described by a 3-compartment model, whereas a 2-compartment model was sufficient to describe the disposition of M6G. After administration of morphine, a fraction (F_{mp}) of the morphine clearance (CL_M) accounted for the formation of M6G.[4] The link between morphine and M6G was located between the 2 central compartments. A delay between the disappearance of morphine from plasma and the reappearance of the molecule as M6G in plasma was modeled by introduction of a "metabolism" compartment with first-order elimination between the central compartments of morphine and M6G. This significantly improved the fit, and fixing the first-order rate constant (λ_M) at a value of 100 (ie, effectively eliminating the delay) resulted in an increase in the objective function of NONMEM of 85. We had also noticed this delay with λ_M in our previous approach to pharmacokinetic modeling of morphine and M6G.[4] We used the following model A to describe the pharmacokinetics of both morphine and M6G, including the formation of M6G:

$$[dA(1)]/dt = I_M - CL_M \cdot C(1) - Q2_M \cdot C(1) + Q2_M \cdot C(2) - Q3_M \cdot C(1) + Q3_M \cdot C(3)$$

$$[dA(2)]/dt = Q2_M \cdot C(1) - Q2_M \cdot C(2)$$

$$[dA(3)]/dt = Q3_M \cdot C(1) - Q3_M \cdot C(3)$$

$$[dA(4)]/dt = \{I_{M6G}\} - CL_{M6G} \cdot C(4) - Q_{M6G} \cdot C(4) + Q_{M6G} \cdot C(5) + \lambda_M \cdot A(6)$$

$$[dA(5)]/dt = Q_{M6G} \cdot C(4) - Q_{M6G} \cdot C(5)$$

$$[dA(6)]/dt = F_{mp} \cdot CL_M \cdot C(1) - \lambda_M \cdot A(6)$$

in which the numbers denote the compartments (1 to 3, the central and peripheral compartments of morphine; 4 and 5, the central and peripheral compartments of M6G; 6, the metabolism compartment), A denotes the amount of drug, C is the concentration calculated by dividing the amount of drug in a compartment by the volume of distribution (V) of that compartment, CL is the total body clearance, Q represents the intercompartmental clearances, F_{mp} is the fraction of the morphine clearance accounting for the formation of M6G, λ_M is the first-order rate constant of the elimination from the metabolism compartment, and I_M and I_{M6G} are the input functions of morphine and, in the case of direct D_3-M6G administration, of M6G. In our study, we

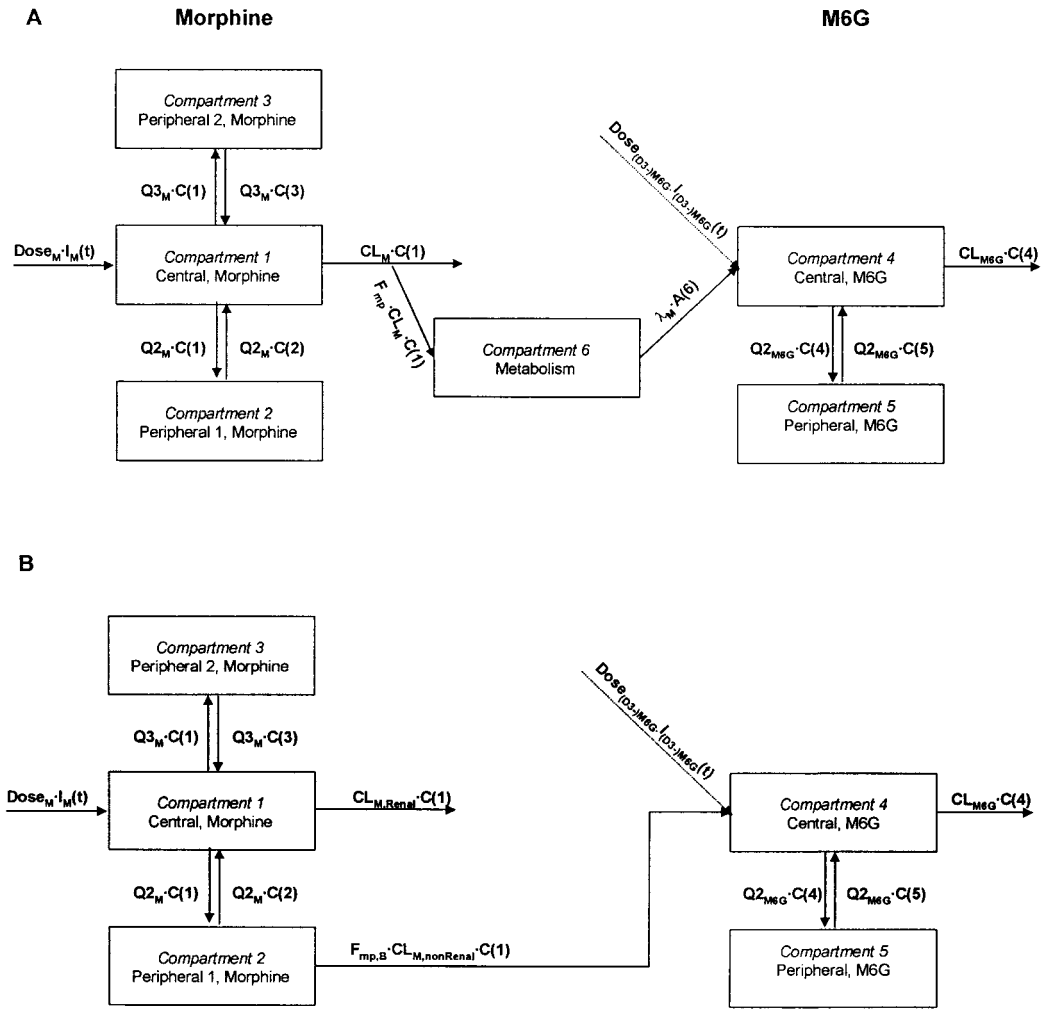

Fig 1. Pharmacokinetic model A **(A)** and pharmacokinetic model B **(B)** of plasma concentration versus time data of morphine and M6G after intravenous administration of morphine [input function $I_M(t)$] and M6G [input function $I_{M6G}(t)$]. M, Morphine; M6G, morphine-6-glucuronide; Q, intercompartmental clearance; C, concentration calculated by dividing the amount of drug in a compartment by the volume of distribution of that compartment; A, amount of drug; numbers 1, 2, and 3, central and peripheral compartments of morphine; numbers 4 and 5, central and peripheral compartments of M6G; number 6, metabolism compartment; CL, total body clearance; F_{mp}, fraction of the total morphine clearance that accounts for the formation of M6G (model A); $I_{(D3-)M6G}(t)$, input function for the deuterium-labeled M6G; λ_M, first-order rate constant of the elimination from the metabolism compartment; $CL_{M,renal}$, renal clearance of morphine; $F_{mp,B}$, fraction of the nonrenal morphine clearance for the formation of M6G (model B); $CL_{M,nonrenal}$, nonrenal clearance of morphine.

measured the exact duration of drug injection, which was regarded as a short-term infusion (average duration of 102 seconds). This was not completely accurate because it regarded the intravenous injection as an input at a constant rate, which was probably not exactly the case. On the other hand, regarding the input as a simple bolus injection (and not taking any measurable time) implied a possibly greater inaccuracy of the description of the drug input. Therefore we described the input as follows:

$$I_M(t) = \text{Infusion rate} \cdot [\text{Heaviside}(\tau - t)]$$

The Heaviside(x) step function is defined as 0 for $x < 0$ and as 1 for $x > 0$. To emphasize that M6G is not usually administered together with morphine, its direct input by intravenous injection is shown in parentheses in the model presentations.

Model B (Fig 1, *B*) is an alternative model that assigns the transfer of morphine to M6G between the peripheral compartment of morphine and the central compartment of M6G. For morphine, therefore, input took place in the central compartment but output took place mainly from a peripheral compartment. However, about 10% of morphine was eliminated unchanged through the kidney.[10] Because this was mainly a result of filtration, it appeared to be more reasonable to assign the morphine excretion to the central compartment. We lacked actual urine data; therefore we assigned published values for the renal excretory clearance of morphine[11] of 2.3 ± 0.6 mL/min/kg to the parameter value and interindividual variability, respectively. The reported standard deviation was transformed into the ϵ variable, which characterized the interindividual variability as described in the NONMEM User's Guide[6] (release V 1.1, part V, page 86). The renal clearance value of morphine ($CL_{M,renal}$) was reported[11] as it related to the subjects' weight, without checking that covariate association. We therefore multiplied the reported value of 2.3 ± 0.6 mL/min/kg with the body weight of each subject, calculated the median of those values, and entered that value as a fixed value of $CL_{M,renal}$ into the model. By applying the same goodness-of-fit assessment throughout the data analysis, we checked whether or not the renal clearance was actually related to the subjects' weight. We checked the need of a variable accounting for the interindividual variance of $CL_{M,renal}$ in a similar manner.

In the alternative model, the delay between the disappearance of morphine from plasma and the reappearance of the molecule as M6G in plasma (as occurred in the first model) was not observed; therefore a metabolism compartment was not necessary. This was observed during initial exploratory data analyses. Thus, the following calculations were used for alternative model B:

$$[dA(1)]/dt = I_M - CL_{M,renal} \cdot C(1) - Q2_M \cdot C(1) + Q2_M \cdot C(2) - Q3_M \cdot C(1) + Q3_M \cdot C(3)$$

$$[dA(2)]/dt = Q2_M \cdot C(1) - Q2_M \cdot C(2) - CL_{M,nonrenal} \cdot C(2)$$

$$[dA(3)]/dt = Q3_M \cdot C(1) - Q3_M \cdot C(3)$$

$$[dA(4)]/dt = \{I_{M6G}\} - CL_{M6G} \cdot C(4) - Q_{M6G} \cdot C(4) + Q_{M6G} \cdot C(5) + F_{mp,B} \cdot CL_{M,nonrenal} \cdot C(2)$$

$$[dA(5)]/dt = Q_{M6G} \cdot C(4) - Q_{M6G} \cdot C(5)$$

in which $CL_{M,renal}$ and $CL_{M,nonrenal}$ denote the renal and nonrenal clearances of morphine. The other variables and compartment numbers are labeled as shown in equation for model A. Note that F_{mp} in model A is the fraction of the total clearance that accounts for the formation of M6G, whereas in model B $F_{mp,B}$ is the respective fraction of the nonrenal morphine clearance only.

Assignment of all morphine output data in model B to the peripheral compartment of morphine (ie, not distinguishing between renal excretion and nonexcretory elimination) resulted in a good fit that was similar to that obtained with the present model B. However, in that case it would have been difficult to explain why renal elimination occurred from the central compartment of M6G but from a peripheral compartment of morphine. Because of this interpretation difficulty, we chose the present model B. Other alternative models, such as one that assigned the morphine-to-M6G link to the peripheral compartments of both morphine and M6G or one that assigned the output of both morphine and M6G to the peripheral compartments, resulted in fits that were not as good and therefore are not presented.

We assessed the prediction performance of the models by applying it to independent data available from intravenous morphine administration to 8 healthy volunteers from another study at another study center (Mundipharma GmbH, Limburg/Lahn, Germany). Those 8 men had the following demographic parameters: mean age, 23.9 ± 0.7 years; mean body weight, 80.1 ± 8.8 kg; mean height, 183.6 ± 5.6 cm; and mean BSA, 2.02 ± 0.11 m^2. The percent prediction error (PE) was calculated for each data point as follows:

$$PE = [(C_{predicted} - C_{observed})/C_{observed}] \cdot 100$$

The median prediction error that measured the bias and the median absolute prediction error that measured the inaccuracy were calculated for each individual. The mean values of the individual median prediction error and the median absolute prediction error were subsequently calculated and compared between the different models.

Finally, the amount of M6G formed from morphine was computed from the values for area under the plasma concentration versus time curve (AUC) of D_3-M6G and the M6G observed after administration of morphine:

$$Amount_{M6G} = (Dose_{D3M6G} \cdot AUC_{M6G})/AUC_{D3M6G}$$

The AUC values were calculated by use of the mixed log-linear trapezoidal rule and extrapolation to infinity

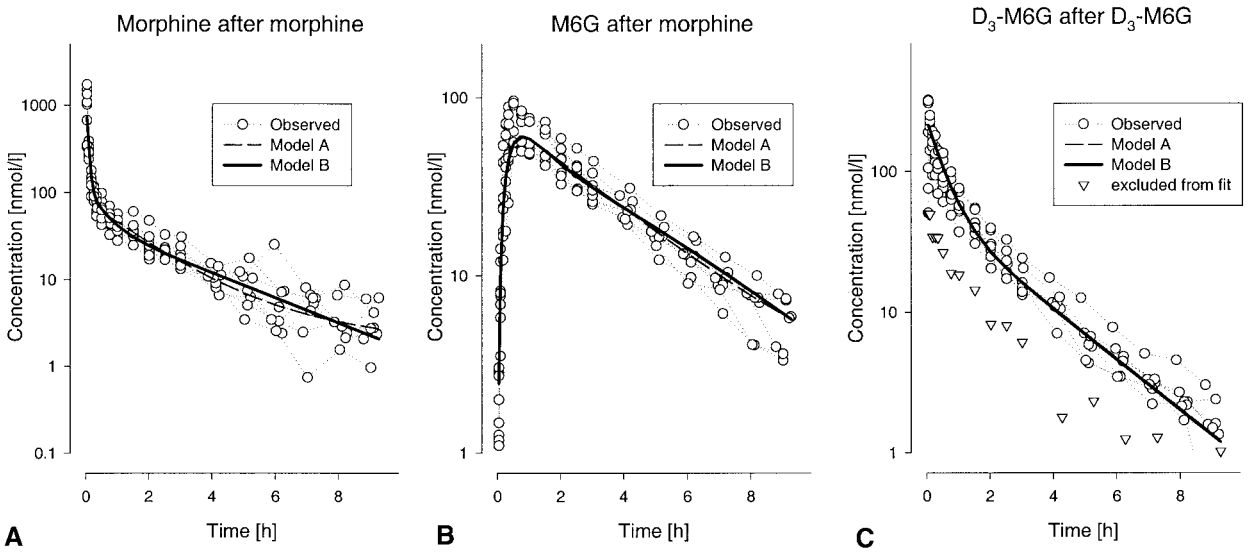

Fig 2. A, Observed plasma concentrations of morphine *(circles)* after intravenous injection of morphine. **B,** Observed plasma concentrations of morphine *(circles)* after intravenous injection of M6G after intravenous injection of morphine. **C,** Observed plasma concentrations of D_3-M6G *(circles)* after intravenous injection of D_3-M6G. The D_3-M6G data from one subject *(triangles)* were excluded from the analyses because of a highly probable dosing error with the D_3-M6G. *Broken lines,* Model A; *solid lines,* model B. M6G, Morphine-6-glucuronide; D_3-M6G, deuterium-labeled M6G.

with Kinetica software (version 3.1, InnaPhase, Philadelphia, Pa). The AUC values of morphine and M3G were also calculated for data consistency checks.

RESULTS

All subjects completed the study, and no subjects had any side effects that required medical intervention. The D_3-M6G AUC for subject 2 was only one fourth of the median of all other subjects, whereas his AUC values for morphine, M3G, and M6G formed from morphine were well within the range of those of the other subjects. The calculation of the amount of M6G formed from morphine on the basis of AUC values resulted in a value of more than 50% for this subject, which was very unlikely and in all probability was caused by a dosing error of D_3-M6G. The D_3-M6G plasma concentrations of this subject were therefore excluded from the analyses.

The median AUC from time zero to infinity was 206.3 nmol/L · h (range, 158.3 to 283.4 nmol/L · h) for D_3-M6G and 225.3 nmol/L · h (range, 182.2 to 337.1 nmol/L · h) for morphine. The AUC of M6G formed from morphine was 247.5 nmol/L · h (range, 188.7 to 321.6 nmol/L · h) and the AUC of M3G formed from morphine was 1597.8 nmol/L · h (range, 1134.3 to 2104.7 nmol/L · h). The extrapolated areas were less than 3% for D3-M6G, less than 11% for morphine, less than 12% for M6G, and less than 18% for M3G. The median amount of M6G formed from morphine was 12.6% of the morphine dose. The median M3G/morphine ratio was 6.6 (range, 3.9 to 10.9), and the median M6G/morphine ratio was 1.1 (range, 0.8 to 1.4).

Plasma concentration versus time curves of morphine and M6G and the population estimates obtained with models A and B are shown in Fig 2. In model A, BSA was linked to the intercompartment clearance between the central and the fast peripheral compartments of morphine by a multiplicative model. Other covariates did not significantly improve the fit with model A and were therefore not part of the final model. In model B, BSA was linked to the volume of the slow peripheral compartment by a multiplicative model. Weight could not be proven to be a covariate of the renal clearance. In addition, assignment of an interindividual variance of the renal clearance was not necessary, as judged by goodness-of-fit criteria. The values of the structural parameters and those of the interindividual variances for both pharmacokinetic models are given in Table II.

Fig 3 shows the prediction of morphine and M6G

Table II. Parameters of the population pharmacokinetic models for morphine and M6G

	Model A		*Model B*	
Parameters (units)	*Fixed effects: Population central values (and % SEE)*	*Random effects (%CV)*	*Fixed effects: Population central values (and % SEE)*	*Random effects (%CV)*
CL_M (L/h)	75.3 (9)	20.2	—	—
$CL_{M,renal}$ (L/h)	—	—	9.6 (—)	0
$CL_{M,nonrenal}$ (L/h)	—	—	134 (13)	30.6
$V1_M$ (L)	17.8 (25)	30.2	15.5 (26)	32.1
$Q2_M$ (L/h)	136 (12)	0	162 (8)	0
$V2_M$ (L)	87.3 (8)	0	92.6 (12)	0
$Q3_M$ (L/h)	$19.5 \cdot (BSA/1.83^*)^4$ (SEE, 9% for $Q3_M$, 23% for the exponent)	22.1	73.9 (21)	0
$V3_M$ (L)	199 (10)	0	$106 \cdot (BSA/1.83^*)^{2.3}$ (SEE, 12% for $V3_M$, 17% for the exponent)	0
CL_{M6G} (L/h)	9.9 (7)	13.5	9.9 (7)	13.78
$V4_{M6G}$ (L)	9.5 (8)	22.9	9.5 (8)	26.1
Q_{M6G} (L/h)	5.8 (24)	0	5.6 (25)	0
$V5_{M6G}$ (L)	7.1 (14)	14.3	7 (14)	12.4
F_{mp}	0.137 (6)	10.6	—	—
$F_{mp,B}$	—	—	0.14 (14)	11.7
λ_M [h^{-1}] (h^{-1})	3.5 (17)	0	—	—

The volumes (V) are numbered as the respective compartments. When a parameter was not part of the model, the corresponding cell of the table contains a line.

M6G, Morphine-6-glucuronide; %SEE, percent coefficient of variation of the population parameter estimate; %CV, percent coefficient of variation CL, total body clearance; M, morphine; $CL_{M,renal}$, renal clearance of morphine; $CL_{M,nonrenal}$, nonrenal clearance of morphine; Q, intercompartmental clearance; F_{mp}, fraction of the total morphine clearance that accounts for the formation of M6G (model A); $F_{mp,B}$, fraction of the nonrenal morphine clearance for the formation of M6G (model B); λ_M, first-order rate constant of the elimination from the metabolism compartment.

Numbers 1, 2, and 3, central and peripheral compartments of morphine; numbers 4 and 5, central and peripheral compartments of M6G; number 6, metabolism compartment.

*The median body surface area (BSA) had a value of 1.83.

plasma concentrations for the 8 independent subjects from whom plasma concentrations were available. Model B predicted the morphine plasma concentrations slightly better than model A, as judged by the values of the mean of the median prediction errors (morphine, 14.8% with model A and −2.4% with model B; M6G, −3.4% with models A and B). The accuracy of the prediction of morphine plasma concentrations was slightly better with model B than with model A, as reflected in the higher mean of the absolute median prediction errors (model A, 28.4%; model B, 19.7%). The performance of the models in predicting the plasma concentrations of M6G was equal (model A, 19.8%; model B, 19.9%).

DISCUSSION

The pharmacokinetic models and their structural parameters, including covariates as estimated with NONMEM, were able to predict independent data obtained from a comparable study population. Our analysis was aimed at improving the pharmacokinetic bases for investigations with morphine in healthy volunteers. Apart from the selected population of healthy young volunteers, we have no indication that the present model parameters will predict morphine and M6G concentrations in considerably younger or older patients or in patients with liver or kidney dysfunction or who otherwise differ from the present population. More covariates (other than BSA) are likely to be identified in specific patient populations, such as kidney function and M6G clearance in patients with kidney failure[12] or liver function and morphine clearance in patients with liver disease.[13]

Model A is an adaptation of the population modeling of our previously developed model for morphine and M6G.[4] However, the prediction performance of the current estimated parameters was much better than that of the previous model, for which the prediction bias for morphine and M6G was −21.3% and −41.2%, respectively, and the mean value of the median absolute prediction errors was 23.8% and 42.6% for morphine and M6G, respectively. This may be a result of the

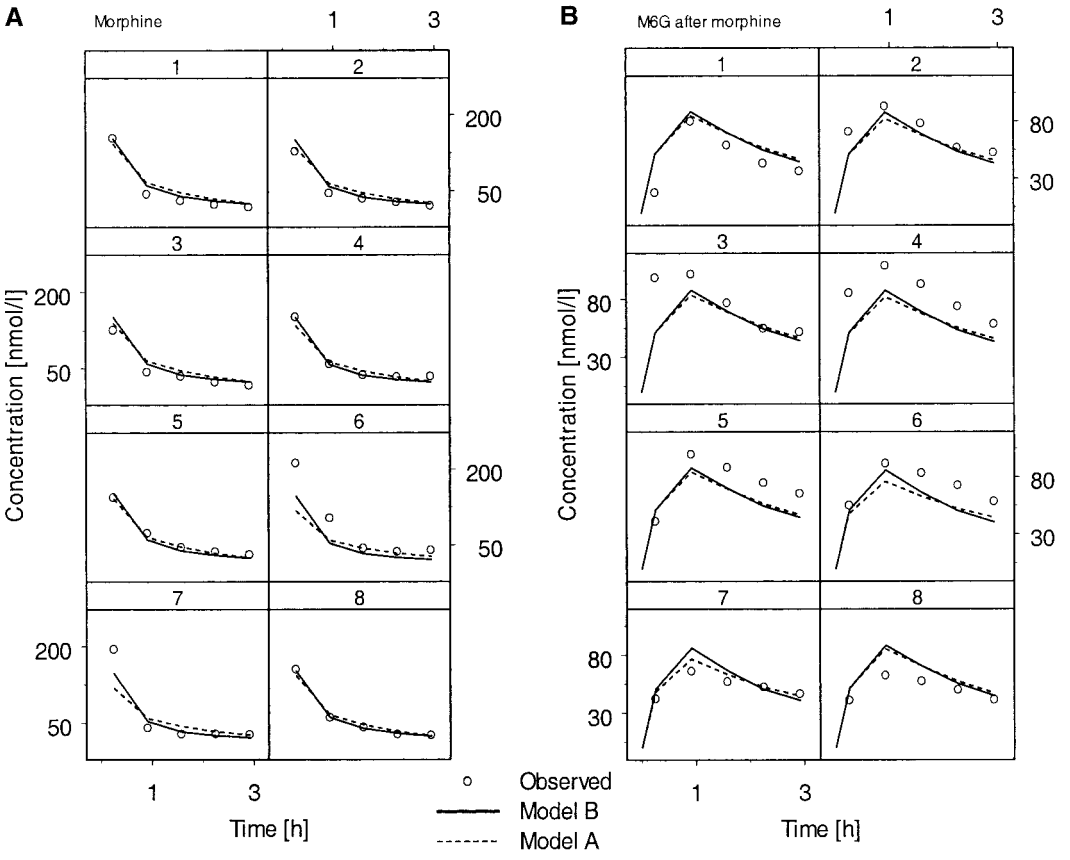

Fig 3. Observed morphine **(A)** and M6G **(B)** plasma concentrations *(circles)* in 8 subjects (subject numbers 1 to 8) different from those in whom the present pharmacokinetic parameters were obtained. *Broken lines,* Predictions made with model A; *solid lines,* predictions made with model B.

present LC-MS-MS analytical method with a quantification limit that was one magnitude lower compared with the previously used HPLC method.[4] This made it possible to identify a 3-compartment disposition of morphine in contrast to the previously applied 2-compartment model. Another reason for the better prediction performance of the present models may be the better fitting algorithm used by NONMEM compared with that utilized by the Scientist fitting software (MicroMath Inc, Salt Lake City, Utah) used previously. Finally, the population approach might have led to better predictive results than the 2-stage approach used previously.

Model A had the advantage of being rather standard (ie, it described the morphine and M6G dispositions by conventional 3- and 2-compartment models, respectively). This made its parameters suitable for use with standard equations to calculate dosing regimens for a specific target concentration, or they could be provided to computerized infusion programs such as Stanpump.[14] The delayed appearance of M6G after administration of morphine was explained previously by a trapping effect that prolonged the transit time of the generated M6G across the site of metabolism (ie, by a longer intracellular residence time of the more hydrophilic metabolite caused by the lower permeability of the cell membrane).[15,16] The mean transit time across the site of metabolism of the generated metabolite M6G (given by $1/\lambda_M$) of 17.2 minutes (0.29 hours) is similar to that calculated previously.[4] It is consistent with the time to peak concentration of the appearance of the generated M6G after bolus intravenous injection of morphine (0.6 hours).[11] However, the hypothesis of a transit time of M6G is not based on experimental evidence. Considering the purely theoretical nature of this explanation, model B was developed as an alternative modeling approach to the delayed appearance of M6G in plasma after administration of morphine. It fitted the

data as well as model A, and its prediction performance was even slightly better than that of model A.

A disadvantage of model B in our present study was the need for external data on renal excretory morphine clearance. $CL_{M,renal}$ was unidentifiable when left free to fit. From a pure fitting point of view, the separate $CL_{M,renal}$ parameter that described excretory output from the central compartment was not necessary. Assignment of all morphine output to a peripheral compartment, without separation of excretory and nonexcretory clearance, resulted in similarly good fits. The parameters estimated with that model version differed from those presented for model B by 0% to 13%, except for the morphine clearance parameter, which differed by 20% because it now described the total clearance. In contrast, assigning the output of M6G to the peripheral compartment visibly and statistically significant worsened the fit. Because M6G is eliminated almost exclusively through the kidney, this pointed toward an renal excretion from the central compartment. For consistency reasons, we therefore preferred the model B with renal excretion from the central compartment to a model version with output from a peripheral compartment only.

The fits and predictions were identical with both model versions. The disadvantage of substitution of the renal morphine clearance value from the literature is easily overcome when urine is collected but was not included in this study because use of model B had not been anticipated when the investigation was planned. A second disadvantage of model B is that it is nonstandard for the morphine disposition and, consequently, the standard equations for dose calculations do not apply and software for computerized infusion does not support model B.[17] Finally, we must note that model B, with a morphine-to-M6G link that starts from a peripheral compartment of morphine, is also not backed by experimental evidence (ie, it is not a more likely explanation of the delayed appearance of M6G in plasma than the metabolic transit time hypothesis). With respect to M6G disposition, however, model B is not different from model A, as reflected in the similar parameter estimates for M6G that were different from those estimated with model A by less than 5%.

This analysis identified a 3-compartment model for morphine (specifically with model A), probably because of the lower limit of quantification of the present LC-MS-MS analytical method compared with the previously used HPLC analytic method.[4] As discussed previously,[11] the neglect of a the third compartment in our previous analysis[4] probably overestimated the clearance of morphine. This is likely to be the reason for the present estimate of 75.3 L/h for CL_M (model A) compared with 130 L/h for the former 2-compartment model.[4] By reparameterization of the present parameters, the half-life of the third terminal phase of the morphine plasma concentration versus time curve is 9 hours, which is much closer to the 15 hours previously concluded from urine data[11] than the 1.7 hours that resulted from the 2-compartment model approach.[4] The estimated half-life of the third exponential term of 9 hours is as long as the sampling time. However, the effective half-life of morphine was much shorter than that suggested by the third exponential. The concept of effective half-life is explained elsewhere.[18] It determines the half-life of a drug not exclusively from the last identified exponent of the disposition function but includes all exponents into the calculation, weighted according the relative contribution of each curve section to the elimination of the drug. In brief, after the disposition function was reparameterized into coefficients (α_i) and exponents (λ_i), the fractions (F_i) of the drug elimination associated with each of the 3 exponents can be calculated by the following:

$$F_i = \frac{\alpha_i/\lambda_i}{\sum_{i=1}^{3} \alpha_i/\lambda_i}$$

in which i is the number of the exponent. The effective half-life ($t_{½,eff}$) is then calculated as follows:

$$t_{1/2,eff} = \ln(2) \cdot \sum_{i=1}^{3} \frac{F_i}{\lambda_i}$$

The resulting values of effective half-life were 2.8 hours for morphine and 1.2 hours for M6G. When 5 half-life values were considered as an optimum sampling period, 9 hours were much closer to that optimum with the effective half-life than with the half-life suggested by the last exponent.

The estimated body clearance of morphine in this study that corresponded to an average of 18.5 mL/min/kg (model A) was then much closer to the value of 21.1 mL/min/kg estimated by Hasselström and Säwe[11] from urine data than to the previously obtained value of 32.7 mL/min/kg.[4] The lower clearance of morphine compared with the probably overestimated values that resulted from the 2-compartment analysis also explains why the value of F_{mp} of 0.14 was higher than the previously calculated value of 0.0755.[4] When a part of the morphine clearance accounts for the formation of M6G, that part must be higher when the clearance becomes lower, assuming that a certain amount of M6G

Table III. Goodness-of-fit parameters

	Model A				*Model B*			
	Fixed effects: Population central values				*Fixed effects: Population central values*			
Parameters	*All*	*Morphine*	*M6G*	*D_3-M6G*	*All*	*Morphine*	*M6G*	*D_3-M6G*
ε (%CV)	23.3				24.3			
MDAWR	18	22	18	14	17	20	18	14
MDWR	−2	−1	−3	−5	−4	−1	−4	−5

%CV, Percent coefficient of variation; M6G, morphine-6-glucuronide; D_3-M6G, deuterium-labeled M6G; MDAWR, median absolute weighted residuals, based on population prediction; MDWR, median weighted residuals, based on population prediction; ε, variable with a mean of zero and a variance of σ^2 that describes a proportional error in the logarithmic domain.

is formed from morphine, regardless of how this formation is modeled. However, the fraction of 13.7% and 14% of the clearance that accounted for M6G formation estimated with model A and B, respectively, agreed well with the value of 12.6% estimated with the AUC approach, and it was close to the previously estimated values of about 10%.[10,19] In contrast to the clearance of morphine, the estimates of the M6G clearance that corresponded to 2.4 mL/min/kg (models A and B) were close to the previously estimated value of 2.18 mL/min/kg.[4] This was probably because, unlike morphine, the disposition function of M6G was also a 2-compartment function as it was used previously.[4]

We ran the models with the complete data set (ie, without exclusion of the D_3-M6G data from the subject in whom the D_3-M6G had an unlikely small value). The effect of the exclusion on the morphine disposition parameters was small, with changes in the typical parameter values of not more than 2%. It was more pronounced on the M6G disposition parameters and on the value of F_{mp}, which were up to 25% smaller with the reduced data set. Furthermore, the interindividual variability of the M6G disposition parameters was about twice as high in the complete data set compared with the reduced set. The reduced data set was better fitted than the full data set, judged by the median absolute weighted residuals, which were 0.18 and 0.17 for models A and B, respectively, for the currently used data set (Table III) and 0.21 and 0.20 for models A and B, respectively, for the full data set. The effect occurred mainly on the D_3-M6G concentrations, with median absolute weighted residuals of 0.14 for the reduced data set (Table III) and 0.20 and 0.21 for models A and B, respectively, for the complete data set (regardless of whether the median absolute weighted residuals were calculated for all 8 subjects or for the 7 other subjects only). However, the prediction performance of the pharmacokinetic parameters was similar, regardless of whether the full or reduced data set was used for the estimates.

This study focused on obtaining predictive pharmacokinetic parameters for the clinically active compounds of morphine and M6G; it was not designed to assess the pharmacokinetics of M3G, the main metabolite of morphine. Direct administration of M3G analogously to D_3-M6G would have been required to obtain the disposition function of M3G. Experiments in laboratory animals have repeatedly suggested that M3G has effects that are opposite to those of morphine and M6G (ie, anti-analgesic and neuroexcitatory effects),[20-22] which do not appear to be mediated by opioid receptors.[23] However, no anti-morphine effects of M3G were found in an approximately similar number of investigations.[24-27] The few instances of direct administrations of M3G to humans showed that M3G did not modified the effects of morphine and had no other effects in healthy volunteers.[28,29] In those studies, M3G was administered at doses up to 30 mg/70 kg, which is approximately 3 times more than the amount of M3G that results from administration of 10 mg morphine, assuming that 57%[11] of the morphine is metabolized to M3G. Furthermore, accumulation of M3G in patients with renal failure is proportional to M6G and was found at concentrations of 10,220 nmol/L,[30] 12,000 nmol/L,[31] and 13,790 nmol/L.[12] However, the great majority of reported clinical problems in those patients related to excessive sedative/depressing opioid effects, which contrasts with the assumed anti-morphine/anti-M6G effect of M3G. Only a single case of allodynia after morphine administration was hypothesized to have been caused by M3G.[32] Thus most evidence currently suggests that M3G has little clinical effect over a wide dose range.

In conclusion, we have provided models of the pharmacokinetics of morphine that include M6G and that, together with their parameter values, can predict the plasma concentrations of both compounds with acceptable accuracy in healthy young volunteers. Either model (A or B) may serve as pharmacokinetic basis for future studies of morphine and M6G in healthy volun-

teers. The parameters of model A may be used as a basis of morphine administration for computerized infusions or other dosing regimens that aim at predefined target concentrations of morphine or M6G in specific groups of healthy young volunteers, such as those often recruited for phase I studies that include morphine administration.

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References

1. Reidenberg MM. Clinical pharmacology: the scientific basis of therapeutics. Clin Pharmacol Ther 1999;66:2-8.
2. Paul D, Standifer KM, Inturrisi CE, Pasternak GW. Pharmacological characterization of morphine-6 beta-glucuronide, a very potent morphine metabolite. J Pharmacol Exp Ther 1989;251:477-83.
3. Kamata O, Watanabe S, Ishii S, Ueki S, Oguri K, Ida S, et al. Analgesic effect of morphine glucuronides [abstract]. In: Proceedings of the 89th Meeting of the Pharmacology Society of Japan. 1969. p. 443.
4. Lötsch J, Weiss M, Kobal G, Geisslinger G. Pharmacokinetics of morphine-6-glucuronide and its formation from morphine after intravenous administration. Clin Pharmacol Ther 1998;63:629-39.
5. Lötsch J, Skarke C, Schmidt H, Grösch S, Geisslinger G. The transfer half-life of morphine-6-beta-glucuronide from plasma to effect site assessed by pupil size measurement in healthy volunteers. Anesthesiology 2001;95:1329-38.
6. Boeckmann AJ, Sheiner LB, Beal SL. NONMEM user's guide. San Francisco: Univ. of California, San Francisco; 1994.
7. Kataria BK, Ved SA, Nicodemus HF, Hoy GR, Lea D, Dubois MY, et al. The pharmacokinetics of propofol in children using three different data analysis approaches. Anesthesiology 1994;80:104-22.
8. Dubois D, Dubois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 1916;17:863-71.
9. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
10. Lötsch J, Weiss M, Ahne G, Kobal G, Geisslinger G. Pharmacokinetic modeling of M6G formation after oral administration of morphine in healthy volunteers. Anesthesiology 1999;90:1026-38.
11. Hasselström J, Säwe J. Morphine pharmacokinetics and metabolism in humans: enterohepatic cycling and relative contribution of metabolites to active opioid concentrations. Clin Pharmacokinet 1993;24:344-54.
12. Hasselström J, Berg U, Lofgren A, Säwe J. Long lasting respiratory depression induced by morphine-6-glucuronide? Br J Clin Pharmacol 1989;27:515-8.
13. Hasselström J, Eriksson S, Persson A, Rane A, Svensson JO, Säwe J. The metabolism and bioavailability of morphine in patients with severe liver cirrhosis. Br J Clin Pharmacol 1990;29:289-97.
14. Shafer SL, Varvel JR, Aziz N, Scott JC. Pharmacokinetics of fentanyl administered by computer-controlled infusion pump. Anesthesiology 1990;73:1091-102.
15. Weiss M, Stedtler C, Roberts MS. On the validity of the dispersion model of hepatic drug elimination when intravascular transit time densities are long-tailed. Bull Math Biol 1997;59:911-29.
16. Evans AM. Membrane transport as a determinant of the hepatic elimination of drugs and metabolites. Clin Exp Pharmacol Physiol 1996;23:970-4.
17. Vaughan DP. On the transformation of compartmental systems and the indeterminacy of the volume of distribution. J Pharm Sci 1989;78:1045-50.
18. Rowland M, Tozer TN. Distribution kinetics, clinical pharmacokinetics. 3rd ed. Philadelphia: Lea and Febiger; 1995. p. 313-39.
19. Hasselström J, Alexander N, Bringel C, Svensson JO, Säwe J. Single-dose and steady-state kinetics of morphine and its metabolites in cancer patients: a comparison of two oral formulations. Eur J Clin Pharmacol 1991;40:585-91.
20. Gong QL, Hedner T, Hedner J, Bjorkman R, Nordberg G. Antinociceptive and ventilatory effects of the morphine metabolites: morphine-6-glucuronide and morphine-3-glucuronide. Eur J Pharmacol 1991;193:47-56.
21. Bartlett SE, Cramond T, Smith MT. The excitatory effects of morphine-3-glucuronide are attenuated by LY274614, a competitive NMDA receptor antagonist, and by midazolam, an agonist at the benzodiazepine site on the GABAA receptor complex. Life Sci 1994;54:687-94.
22. Halliday AJ, Bartlett SE, Colditz P, Smith MT. Brain region–specific studies of the excitatory behavioral effects of morphine-3-glucuronide. Life Sci 1999;65:225-36.
23. Löser SV, Meyer J, Freudenthaler S, Sattler M, Desel C, Meineke I, et al. Morphine-6-O-beta-D-glucuronide but not morphine-3-O-beta-D-glucuronide binds to mu-, delta- and kappa-specific opioid binding sites in cerebral membranes. Naunyn Schmiedebergs Arch Pharmacol 1996;354:192-7.
24. Gardmark M, Karlsson MO, Jonsson F, Hammarlund-Udenaes M. Morphine-3-glucuronide has a minor effect on morphine antinociception: pharmacodynamic modeling. J Pharm Sci 1998;87:813-20.
25. Ouellet DM, Pollack GM. Effect of prior morphine-3-glucuronide exposure on morphine disposition and antinociception. Biochem Pharmacol 1997;53:1451-7.
26. Bian JT, Bhargava HN. Effects of morphine-3-glucuronide on the antinociceptive activity of peptide and nonpeptide opioid receptor agonists in mice. Peptides 1996;17:1415-9.
27. Suzuki N, Kalso E, Rosenberg PH. Intrathecal morphine-

3-glucuronide does not antagonize spinal antinociception by morphine or morphine-6-glucuronide in rats. Eur J Pharmacol 1993;249:247-50.
28. Penson RT, Joel SP, Bakhshi K, Clark SJ, Langford RM, Slevin ML. Randomized placebo controlled trial of the activity of the morphine glucuronides. Clin Pharmacol Ther 2000;68:667-76.
29. Penson RT, Joel SP, Clark S, Gloyne A, Slevin ML. Limited phase I study of morphine-3-glucuronide. J Pharm Sci 2001;90:1810-6.
30. Osborne RJ, Joel SP, Slevin ML. Morphine intoxication in renal failure: the role of morphine-6-glucuronide. Br Med J (Clin Res Ed) 1986;292:1548-9.
31. Ashby M, Fleming B, Wood M, Somogyi A. Plasma morphine and glucuronide (M3G and M6G) concentrations in hospice inpatients. J Pain Symptom Manage 1997;14:157-67.
32. Heger S, Maier C, Otter K, Helwig U, Suttorp M. Morphine induced allodynia in a child with brain tumour. BMJ 1999;319:627-9.