

Probenecid Interacts with the Pharmacokinetics of Morphine-6-glucuronide in Humans

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Background: Evidence obtained from porcine cell cultures and experiments in laboratory animals indicates that transmembrane transporters may play a role in the distribution of the active morphine metabolite morphine-6-glucuronide (M6G). This was evaluated in a study in healthy volunteers.

Methods: Ten subjects received an intravenous M6G infusion for 30 min at a dosage of 0.5 mg/kg body weight, leading to M6G plasma concentrations approximately two to three times higher than those observed with analgesic morphine doses in subjects with normal kidney function. In a randomized, double-blind, three-way crossover fashion, subjects received 800 mg quinidine for inhibition of P-glycoprotein; 500 mg probenecid for inhibition of other transporters, including organic anion transporter peptide, multidrug resistance-related protein, and organic anion transporter families; or placebo 1 h before the start of M6G administration. Plasma concentrations of M6G and pupil size were measured for 7 h.

Results: Probenecid pretreatment resulted in a decrease in the clearance of M6G from 8.3 ± 1.1 l/h to 6.7 ± 1.3 l/h (factor of 0.8; $P < 0.05$ vs. placebo cotreatment). This was paralleled by an increase by a factor of 1.2 of the area under the moitic effect-versus-time curves ($P < 0.05$ vs. placebo). In contrast, quinidine pretreatment had no influence on the pharmacokinetics of M6G.

Conclusions: The active morphine metabolite is subject to transmembrane transport by transporters inhibited by probenecid in humans.

MORPHINE-6-GLUCURONIDE (M6G) is an active metabolite of morphine.¹ It is eliminated *via* the kidney. It accumulates in patients with renal failure who may therefore experience opioid side effects during morphine therapy attributable to increased M6G.² However, in patients with increased serum creatinine, the concentrations of M6G widely overlapped between patients with and without opioid side effects.³ One of the factors contributing to this interpatient variance may be transmembrane transporters, which follows from the acknowledgment that drug distribution between different body compartments is not just a passive process but actively regulated by a variety of transport proteins (for reviews, see Kerb *et al.*,⁴ Fricker and Miller,⁵ Kim,⁶ and Inui *et al.*⁷). Their localizations at the blood-brain bar-

rier, the kidney, or the liver make them potentially important for M6G distribution.

Morphine-6-glucuronide has been found to be a substrate of P-glycoprotein in an *in vitro* model.^{8,9} Because P-glycoprotein forms an outward transporter at the blood-brain barrier,¹⁰ P-glycoprotein inhibition leads to an estimated doubled M6G concentration in the central nervous system.^{8,9} However, the expected increase in the antinociceptive effects of M6G was not observed in P-glycoprotein knockout mice.^{11,12} Moreover, a relevant active transmembrane M6G transport was demonstrated in mice lacking P-glycoprotein,¹³ which strongly suggests that other transporters than P-glycoprotein are involved in M6G distribution as well. Likely candidates are members of the organic anion transporter polypeptide (OATP), the organic anion transporter, and the multidrug resistance-related protein (MRP) families, which all have glucuronides among their substrates.¹⁴⁻¹⁷ In rats, probenecid, which inhibits many of these transporters, increased the concentrations in plasma and the central nervous system nonsignificantly by a factor of 1.3¹⁸ and significantly influenced the blood-brain barrier transport of morphine-3-glucuronide (M3G).¹⁹

Therefore, we investigated the effects of transporter inhibition on the plasma concentrations. Quinidine was used to inhibit P-glycoprotein,²⁰⁻²² and probenecid was administered to inhibit several of the other candidate transporters involved in M6G distribution. Pupil size measurement was instituted as an opioid effect parameter to assess whether an altered M6G distribution has consequences for its central nervous effects.

Materials and Methods

Volunteers and Study Design

Five men and five women with an average age, weight, and height of 27.2 yr, 68.9 kg, and 175.7 cm, respectively, were recruited for this study. The study was open with respect to M6G administration and double blind with respect to the administration of transporter inhibitors. M6G (Lipomed, Arlesheim, Switzerland) was administered as an infusion over 30 min. On the basis of simulations using pharmacokinetic²³ and pharmacokinetic-pharmacodynamic²⁴ models, a dose of 0.5 mg/kg was chosen to produce clearly noticeable but not maximum pupil constriction, which was considered to be best suited to identify a modification in the central nervous effects of M6G by coadministration of transporter inhibitors. One hour before M6G administration, subjects ingested in a double-blind, randomized, cross-over

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fashion 800 mg quinidine, 500 mg probenecid, or placebo. A washout interval of at least 7 days was observed. In a previous study in a similar population, we observed quinidine concentrations of approximately 2 $\mu\text{g/ml}$ after 800 mg oral quinidine,²⁵ which produced clinically relevant effects on the QTc time of the electrocardiogram²⁶ and which had been found to reverse P-glycoprotein-mediated tumor resistance in humans to a relevant degree.²⁷ Probenecid dosing was based on the report that 500 mg probenecid every 6 h resulted in an increase of the plasma concentrations and reduction of the urinary excretion of zidovudine.^{28,29}

The study was conducted according to the Declaration of Helsinki on biomedical research involving human subjects (Somerset West amendment). The University of Frankfurt Medical Faculty Ethics Review Board approved the study protocol, and written informed consent was obtained from all subjects before the study.

Blood samples (4 ml) were drawn before the administration of quinidine or placebo, at 15 and 30 min after M6G administration, and then every half hour until the end of the 7-h observation period. Plasma concentrations of M6G, M3G, and morphine were assayed by liquid chromatography tandem mass spectrometry.³⁰ The lower limit of quantification was 1 ng/ml for morphine and 0.5 ng/ml for M6G and M3G. The coefficient of variation over the calibration range of 0.5 (or 1) to 1,250 ng/ml was less than 5%.

Pupil diameter was assessed using a pupillograph (CIP; Amtech GmbH, Weinheim, Germany). After four initial baseline measurements, pupil size was measured with the start of the M6G infusion every 30 min for approximately 7 h as described previously.^{24,26,30}

Data Analysis

A two-stage data analysis was performed with outcome measures being the areas under the M6G plasma concentration and pupil diameter change-*versus*-time curves (AUCs). In addition, the clearance of M6G (CL_{M6G}) was obtained from Dose/AUC, using the AUC extrapolated to infinity. The average contribution of the extrapolated part of the AUC to the total AUC was $5.8 \pm 2.5\%$, with a maximum of 11%. Maximum M6G plasma concentrations, C_{max} , were read from the data, and the terminal half-life was calculated as \ln^2 divided by the slope of a regression line through the terminal linear segment of the log-transformed M6G concentration-*versus*-time curve. The influence of the comedications on C_{max} , AUC, and CL_{M6G} was evaluated by repeated-measures analysis of variance (rm-ANOVA; between-subjects factor *medication*), followed by Student-Newman-Keuls tests for multiple comparisons (Sigma Stat 3.00; SPSS, Inc., Chicago, IL). In addition, M6G morphine plasma concentrations and pupil diameter changes were evaluated by 2-way rm-ANOVA (factors *medication* and *session*, *i.e.*, observation number), followed by the Student-Newman-

Keuls test for multiple comparisons. The α level was set at 0.05. All results are reported as mean \pm SD.

Plasma concentration-*versus*-time data were also analyzed using a parametric modeling approach with NONMEM (GloboMax LLC, Hanover, MD). The modeling process repeatedly used goodness-of-fit procedures, which were (1) the NONMEM objective function being -2 times the log likelihood and the χ^2 approximation with the number of degrees of freedom equal to the difference in the number of terms between two models (α level, 0.05); (2) the median absolute weighted residuals, calculated as (measured $-$ predicted)/predicted, and the mean of the individual mean absolute weighted residuals; and (3) visual inspection of the fits *versus* observed data.

The pharmacokinetics of M6G were described by a two-compartment model:

where A1 and A2 denote the amount of drug in compartments 1 and 2, respectively; C1 and C2 the respective concentrations obtained by dividing the amount of drug by the volume of distribution of the respective compartment, V1 and V2; I(t) is the input function for M6G characterized by rate and duration of the infusion; CL is the total body clearance, and Q the intercompartmental clearance. Alternative one- and three-compartment models were rejected on the basis of goodness-of-fit judgments.

The influence of quinidine or probenecid administration on the model parameters was assessed as follows: First, it was checked whether a particular parameter varied interindividually. Second, it was checked whether there was an intraindividual interoccasion variability on this parameter. If this was found to be the case, it was determined whether this interoccasion variability was partly explained by the comedication. This was done by multiplying the model parameters found to exhibit intraindividual interoccasion variability with 1 in case of placebo coadministration, with a factor θ_1 in case of quinidine coadministration and with a factor θ_2 in case of probenecid coadministration. For example, the clearance CL in equation 1 was replaced by $CL \cdot 1$ for the placebo condition, $CL \cdot \theta_1$ for the quinidine condition, and factors $CL \cdot \theta_2$ for the probenecid condition. If introduction of θ_1 or θ_2 significantly improved the fit, judged by the likelihood ratio test, then an influence of the respective comedication on M6G distribution was demonstrated. This assumed that the influence of a transporter inhibitor on a model parameter was constant throughout the observation period, thus neglecting that the inhibitor's concentrations varied as a consequence of the inhibitor's own concentration-*versus*-time profile. A log-normally distributed interindividual variance of the model parameters was used: , where P_i is the value of the parameter of the individual, $\theta_{i,TV}$ is the typical value of this parameter in the population, and η is a variable accounting for the interindividual variability, with mean

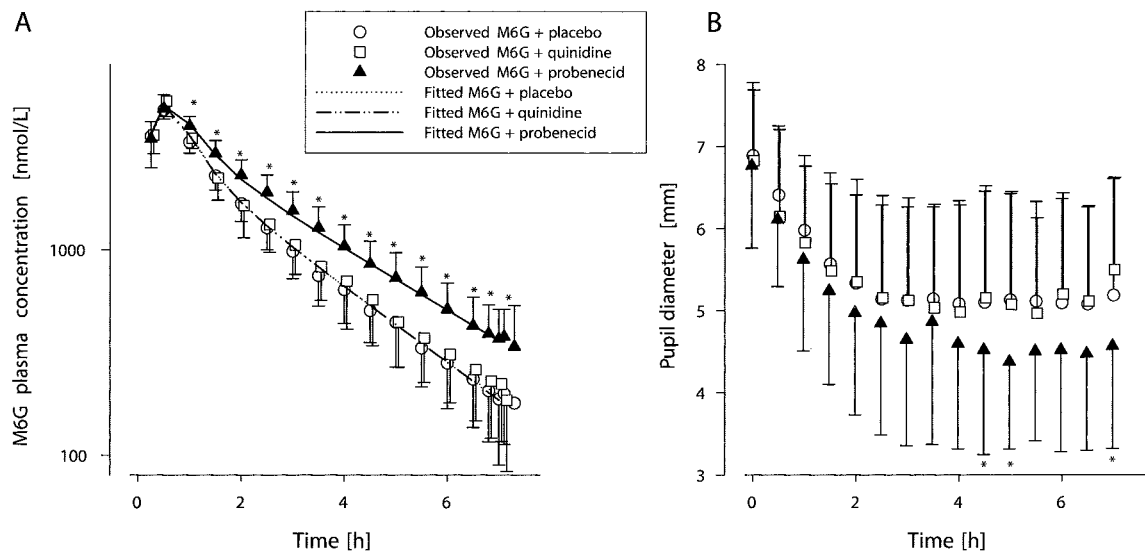


Fig. 1. (A) Observed plasma concentrations of morphine-6-glucuronide (M6G) and the fits obtained by parametric pharmacokinetic modeling after intravenous administration of 0.5 mg/kg M6G with coadministration of 800 mg quinidine, 500 mg probenecid, or placebo to 10 volunteers (* $P < 0.05$). (B) Observed pupil sizes (* $P < 0.05$).

zero and variance ω^2 . The intraindividual interoccasion variability was modeled analogously, as described previously.³¹ An assumption of normal rather than log-normal distribution of parameter values resulted in worse fits and was therefore rejected. The residual error ϵ was modeled using a proportional error model. Calculations were performed using first-order conditional estimation and η - ϵ interaction. Covariates checked for relation with pharmacokinetic structural parameters were age, weight, height, body surface area,³² and sex.

Results

Plasma Concentrations

Probenecid coadministration increased the M6G plasma concentrations (fig. 1A), with significant differences from placebo starting at 1 h after M6G dosing (two-way rm-ANOVA: $P < 0.05$ for factor *medication*, $P < 0.001$ for factor *session*). The AUC was increased 1.26 ± 0.2 times with probenecid coadministration (rm-ANOVA: $P = 0.001$, Student-Newman-Keuls: $P < 0.05$ for probenecid *vs.* placebo) as compared with placebo coadministration, whereas it remained unchanged by quinidine (factor 1.05 ± 0.1). The M6G plasma clearance was significantly influenced by the comedication (rm-ANOVA effect *medication*: $P = 0.001$). It decreased from 8.3 ± 1 l/h under placebo by a factor of 0.82 ± 0.2 to 6.7 ± 1.3 l/h under probenecid coadministration, which was statistically significant (Student-Newman-Keuls: $P < 0.05$ *vs.* placebo; 95% confidence interval [CI] for differences, -2.5 to -0.7 l/h). With quinidine, the clearance of M6G was 8 ± 1.2 l/h, which did not differ statistically significantly from placebo coadministration (95% CI for differences, -1 to 0.4 l/h). The M6G

plasma half-life increased from 1.6 ± 0.2 h under placebo coadministration to 1.8 ± 0.3 h under probenecid coadministration (rm-ANOVA: $P < 0.01$; 95% CI for differences, probenecid to placebo, 0.1 – 0.4 h; Student-Newman-Keuls: $P < 0.05$), whereas it remained unaltered by quinidine coadministration (1.6 ± 0.2 h; $P = 0.4$ *vs.* placebo; 95% CI for differences, -0.1 to 0.2 h). Morphine and M3G were found only in traces in plasma as it had been previously observed.³⁰

The results of the pharmacokinetic modeling analysis are given in table 1. The analysis obtained a significant effect of probenecid administration on the total body clearance of M6G. The NONMEM objective function

Table 1. Parameter Values of Pharmacokinetic Model (Equation 1) to Describe the Plasma Concentrations after Morphine-6-glucuronide Administration

Parameter	Population Central Values (%SEE)	Interindividual Variability, %CV	Intraindividual Interoccasion Variability, %CV
CL, l/h			
+ Placebo	7.6 (4)		
+ Quinidine		13.1	7.6
+ Probenecid	5.9 (7)		
V1, l	9.5·(BSA/1.87) ^{2.1} (SEE 4% for V ₁ and 11% for the exponent)	5.7	—
Q, l/h	5.4 (14)	—	—
V2, l	5.1 (8)	—	—

Variability is given as percent coefficient of variation (%CV).

* E_0 was the actually observed value, not an estimate. The median body surface area (BSA) in the sample was 1.87.

CL = total body clearance; Q = intercompartmental clearance; V1 = volume of distribution of the central compartment; V2 = volume of distribution of the peripheral compartment.

decreased highly significantly ($P < 0.001$) by 31 when assigning θ_2 to the total clearance of M6G, and the intraindividual interoccasion variance decreased from 16.9% to 7.6%, given as percent coefficient of variation. Assigning covariates to additional pharmacokinetic parameters did not further improve the fit. The factor θ_2 was estimated to be 0.8 (95% CI of the estimate, 0.73–0.82), *i.e.*, the clearance of M6G decreased by approximately 20% when probenecid had been coadministered. Therefore, in equation 1, the parameter CL was replaced with $CL \cdot \theta_2$ in case of probenecid coadministration with M6G. In contrast, θ_1 accounting for quinidine coadministration did not significantly improve the fit, and the intraindividual interoccasion variance decreased only from 16.9% to 15.7% coefficient of variation. Finally, the body surface area was a covariate to the central volume of distribution V1, whereas the other covariates tested, including sex, had no influence on the pharmacokinetics of M6G. Figure 1A shows the fits along with the observed data.

Miotic Effects

Coadministration of probenecid but not of quinidine resulted in a more pronounced miosis (fig. 1B), which was statistically significant at three data points covering the time of maximum miotic effects around 4.5 h (two-way rm-ANOVA: $P < 0.05$ for factor *medication*, $P < 0.001$ for factor *session*; Student-Newman-Keuls for probenecid *vs.* placebo: $P < 0.05$). With probenecid coadministration, the AUCs increased by a factor of 1.25 ± 0.3 as compared with placebo coadministration (rm-ANOVA factor medication: $P < 0.05$; *post hoc* Student-Newman-Keuls: $P < 0.05$; 95% CI for differences from placebo coadministration, -4.2 to -0.3 mm · h), whereas AUCs remained unchanged with quinidine coadministration (factor of 1.1 ± 0.3 , 95% CI for differences to placebo, -1.5 to 1.7 mm · h). Therefore, the increase in the AUCs of the miotic effects paralleled the observed effect in the AUCs of the plasma concentration data. This suggested that probenecid had an exclusive effect on the plasma concentrations, with the result that miotic effects just followed the increase in plasma M6G.

Discussion

The results indicate that transmembrane transporter function may affect M6G disposition. The effect of 500 mg probenecid coadministration consisted of a 20% decrease of the M6G clearance. Because M6G is almost exclusively eliminated *via* the kidney (92% of the total amount of M6G³³), involving glomerular filtration, tubular secretion, and reabsorption,³⁴ a likely effect of probenecid was decreasing the renal tubular secretion of M6G, similarly as demonstrated for the action of probenecid on renal excretion of zidovudine^{28,29} or acamprosate.³⁵

The significant effects on the area under the miotic effects-*versus*-time curves indicate that the changes in M6G disposition bear the potential of enhancing the clinical effects of M6G, although currently, the effects were seen only in three data points, *i.e.*, during a short period when maximum miotic effects were observed. Therefore, from the current observations, an important clinically relevant drug interaction cannot be established. The finding is currently more important to attract attention to probenecid sensitive transporters as a possible modulator of the clinical effects of M6G to take a closer look at possible pharmacokinetic interactions of M6G with other drugs for which probenecid was shown to alter their pharmacokinetics, such as zidovudine,^{28,29} adefovir,³⁶ ibuprofen,³⁷ or cephalosporin antibiotics.³⁸ The clinical setting best qualifying for such investigations is morphine therapy in patients with renal failure, in whom M6G accumulates and produces central nervous side effects.^{2,39–41}

Probenecid inhibits several transporters of the MRP, OATP, and organic anion transporter families, which was shown *in vitro* by decreasing the transport at the blood-brain barrier for morphine⁴² or for M3G.⁴³ From interaction of transmembrane transport of M6G with digoxin or the P-glycoprotein inhibitor PSC833 (valsopodar⁴⁴) in P-glycoprotein knockout mice,¹³ from the fact that digoxin has been found to be a substrate of Oatp2^{45,46} and from the observation that PSC833 also interacted with MRP2,⁴⁷ it is concluded that Oatp2 (as already hypothesized¹³) and MRP2 are possible candidate transporters involved in the current effects of probenecid on M6G disposition. OATP-C, MRP2, and P-glycoprotein, which hold an important role for drug disposition, were found to be colocalized in organs such as the kidney, liver, or duodenum,^{48–50} but MRP2 not at the blood-brain barrier.⁵¹ Because they form outward transporters,^{48–50} their inhibition would result in retention of M6G in the body as currently observed after probenecid administration. To obtain a hypothesis regarding which transporter was likely to be involved in decreasing the clearance of M6G by probenecid, we genotyped the participants (with approval of the ethics committee and after having obtained written informed consent) for single nucleotide polymorphisms (SNPs) in the *ABCB1* gene that codes for P-glycoprotein (SNPs 2677G>T(A) and 3435C>T), in the *ABCC2* gene that codes for MRP2 (SNPs -24C>T, 1249G>A, and 3972C>T), and in the *SLCO1B1* gene that codes for OATP-C (SNPs 388A>G and 521T>C). The three carriers of the *SLCO1B1* SNP 521T>C, which causes an amino acid exchange Val174Ala in the OATP-C transporter, had a smaller M6G clearance with probenecid coadministration ($CL_{M6G} = 5.3 \pm 0.7$ l/h) than the seven noncarriers of this particular mutation ($CL_{M6G} = 7.3 \pm 1.1$ l/h). This difference was statistically significant (*t* test: $P = 0.025$; 95% CI for differences, 0.3–3.5 l/h).

Moreover, the clearances of the carriers and noncarriers of that mutation did not overlap. The three carriers had M6G clearances under probenecid cotreatment of 5.3, 4.6, and 6.1 l/h, while the lowest clearance in a noncarrier was 6.3 l/h. None of the other SNPs or resulting haplotypes (the latter in the case that a linkage disequilibrium between single SNPs could be found by means of the EH computer program: J. Ott, Ph.D., Rockefeller University, New York, New York⁵²) influenced any of the parameters of M6G disposition or effects, with all *P* values being well above 0.1. This result could mean that OATP-C is involved in the transmembrane transport of M6G in humans. That an effect of the OATP-C mutation on M6G clearance was only evident with probenecid coadministration does not contradict the hypothesis. It could be seen as a challenge test required to observe the pharmacogenetic effect, analogously to a glucose tolerance test needed for diagnosis of diabetes in individuals without permanently increased blood glucose. Here, the transporter function may have been at the lower range of the distribution, but the differences between genotypes were not great enough to form separate groups. When challenged with probenecid, the compensation capacity of the mutated transporters may have failed earlier so that the mutation carriers were then separated from the nonmutated subjects. However, an involvement of OATP-C in M6G disposition is merely a hypothesis fitting to previous observations from experiments in laboratory animals and supported by the current preliminary pharmacogenetic analysis and by the fact that functional consequences were described for the 521T>C SNP.⁵³ The hypothesis must be tested in a new study using probenecid coadministration with M6G in subjects recruited according to their *SLCO1B1* genotype.

The lack of a quinidine effect given to block P-glycoprotein^{20-22,27} indicated that P-glycoprotein played a minor role in M6G disposition, which contrasts with the expectations raised on the basis of experiments *in vitro*.^{8,54} However, it seems to be in accord with the negative results obtained in P-glycoprotein knockout mice.¹¹⁻¹³ It could also mean that at the blood-brain barrier P-glycoprotein played no important role in the current context. This is similar to the failure to demonstrate P-glycoprotein effects on opioid brain distribution localized at the blood-brain barrier.^{25,55,56} In addition, it could mean that the inhibiting effect of quinidine on P-glycoprotein at the kidney or at the blood-brain barrier was not big enough to alter the disposition of M6G. Quinidine coadministration increased plasma concentrations only of orally and not of systemically administered morphine,^{26,56} which may be related to the much higher local quinidine concentrations to which intestinal P-glycoprotein was exposed in contrast to the comparably lower systemic quinidine concentrations to which P-glycoprotein at other localizations was exposed. It remains to be seen whether more potent P-glycoprotein

modulators than quinidine (for review see Tan *et al.*⁵⁷) interact with the systemic disposition of M6G.

In conclusion, we found that probenecid interacts with the pharmacokinetics of M6G in humans, possibly involving OATP-C transporters.

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