Chapter 6

Arterial and Venous Pharmacokinetics of Morphine-6-Glucuronide and Impact of Sampling Site on Pharmacodynamic Parameter Estimates*

PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) MODELING is an important tool to examine the dynamic behavior of drugs, and because the analysis yields an estimation of drug potency and delay between blood concentration and effect (hysteresis), it allows for an accurate prediction of effect. The hysteresis occurs because of the distributional disequilibrium between the site at which the drug is measured and the site of action (= biophase kinetics).[92] Most contemporary PK-PD models are correctly based on arterial blood samples.[92] However, occasionally, arterial blood samples are not available. This may, for example, occur when it is deemed inappropriate to place arterial catheters and it is assumed that similar results will be obtained by using venous blood samples.

The human ethics committee of our institution expressed its concerns regarding the placement of arterial catheters in healthy volunteers participating in PK-PD studies on long-acting opioids. They reasoned that for some drugs, just a small difference in PD parameter estimates would be obtained when sampling from a venous site and consequently that it was judged unnecessary (and hence unethical given the possibility of serious complications) to place an arterial catheter. We have ample experience with arterial catheter placement and over the years did not encounter any complications. We do agree with them, however, that the consequences of complications such as radial artery occlusion, nerve damage, or pseudo aneurysm of the radial artery are serious and need to be carefully balanced against the gain of obtaining arterial blood samples. In cooperation with the human ethics committee we therefore decided to perform a study in volunteers in which both arterial and venous (from a peripheral site, *i.e.*, the arm) drug

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samples were obtained after IV infusion of the opioid morphine-6-glucuronide (M6G), a drug of long action.[69] The dose chosen, 0.3 mg/kg, causes long-lasting analgesia but only moderate respiratory depression.[69,71,68]

The present study was performed in 3 steps. Initially, we determined the arterial and venous concentrations of M6G after a rapid IV infusion. Next, we built a PK model of the drug distribution between arterial and venous blood. Finally, we performed simulation studies to help us to understand the consequences of the (erroneous) assumption that arterial and venous data are equal and the effect on PD parameter estimates when using venous rather than arterial drug concentrations to drive the effect compartment. We hypothesized that because we were dealing with a slow-acting drug, there is no difference between an arterial sample-based model and a venous sample-based model.

6.1 Methods

6.1.1 Subjects

Seventeen healthy volunteers (9 men, 8 women, ages 19 to 34 years and body mass index <28) participated in the study after approval of the protocol by the local ethics committee and after giving written informed consent. All subjects were asked to refrain from food for at least 8 hours before the start of the study.

6.1.2 Study Design

After arrival in the laboratory, 2 venous catheters (in the left and right cubital veins) and 1 arterial catheter (in the radial artery at the wrist of the nondominant arm) were inserted. One venous catheter was placed for drug infusion; the other venous catheter and the arterial catheter were placed for blood sampling. At t = 0, 0.3 mg/kg M6G was infused IV over 90 seconds. Next, arterial and venous samples were obtained (simultaneously) at times t = 5, 10, 20, 30, 40, 50, 60, 80, 120, 180, 240, 300, 360, and 420 minutes. Plasma was separated within 10 minutes of blood collection and stored at -25°C until analysis. M6G measurement has been described.[71] Briefly, serum was pretreated by protein precipitation with acetonitril; M6G was measured using liquid chromatography with tandem mass spectrometry. The between-days coefficients of variation were 4.1% and 4.0% for 75 and 1800 µg/L, respectively; the within-day coefficients of variation were 0.5% and 2.0%. The quantitation limit was set at 20 ng/mL.

6.1.3 Pharmacokinetic Analysis

The arterial and venous concentration data were analyzed simultaneously. To that end, the arterial data were analyzed first with 2 or 3 compartments. Next, arterial and venous data were analyzed simultaneously with 3 compartments for arterial data and 1 or 2 compartments for venous data. Objective function values were reported for the latter 2 cases, to give an indication for the importance of the second venous compartment. The venous samples are from the periphery, *i.e.*, the forearm. Data analysis was performed with the statistical package NONMEM VI, version 1.2. Model selection (the number of compartments) was based on the goodness-of-fit criterion, *i.e.*, the magnitude of the

decrease in minimum objective function value (MOFV; X^2 test (for nested models): P < 0.01 was considered significant).

For the concentrations in the central and peripheral venous compartments (C_{V_1} and C_{V_2}, respectively) we write (Figure 6.1A)

$$V_{V_1} \cdot dC_{V_1}/dt = CL_{AV} \cdot (C_{A_1} - C_{V_1}) - CL_{V_2} \cdot (C_{V_1} - C_{V_2}) \quad (6.1)$$

$$V_{V_2} \cdot dC_{V_2}/dt = CL_{V_2} \cdot (C_{V_1} - C_{V_2}), \quad (6.2)$$

where V_{V_1} and V_{V_1} are the volumes of the central and peripheral venous compartments, CL_{AV} the arteriovenous (AV) clearance, and CL_{V_2} the central-peripheral venous clearance (see Appendix and Figure 6.1A for details).

6.1.4 Simulation Studies

Simulation studies were conducted to assess the influence of sampling site on estimated PD parameter values and on prediction. The PD model consisted of an inhibitory sigmoid E_{max} model, with a baseline effect of 1 and a minimum effect of 0 (*e.g.*, describing the respiratory effect of M6G):

$$\text{Effect}(t) = 1/\left(1 + (C_e(t)/EC_{50})^{\gamma}\right), \quad (6.3)$$

where where $C_e(t)$ is the effect-site concentration at time t, EC50 the effect-site concentration giving 50% effect, and γ a shape factor (the Hill coefficient). An effect site is postulated. The equilibration rate constant between arterial blood and effect site is k_{e0} with half-life $t_{½,k_{e0}}$. The effect site has to be linked to arterial blood concentrations to obtain correct PD parameter estimates (Figure 6.1). However, when only venous blood samples are available, the effect site may be (incorrectly) linked to venous blood concentrations (Figure 6.1). EC_{50} was set to 500 (a typical value for pain relief from M6G with $t_{½,k_{e0}}$ values ranging from 2 to 6 hours),[68] γ to 1 (as observed in studies on the effect of M6G on minute ventilation [where maximal respiratory depression = apnea] and with $t_{½,k_{e0}}$ values ranging from 1 to 2 hours)[69] or 2.5 (as observed in studies on the effect of M6G on pain relief)4 and $t_{½,k_{e0}}$, as given above. These 3 parameters had lognormal distributions across the population with variance 0.1 (coefficient of variation ≈30%)[69,68] The SE of the additive intraindividual error was set to 0.1.

Study I

The purpose of the first set of simulations was to determine the influence of linking venous blood samples to the effect site on PD parameters. One thousand Monte Carlo simulations were performed to generate PD data with arterial concentrations driving the effect site. These PD data were next fitted using arterial or venous concentration data driving the effect site. Using arterial concentration data should yield parameter estimates close to the ones used for simulation; using venous concentrations might yield estimates that are biased because of the erroneous location of the sampling site. For each simulated data set (n = 1000), the ratios of the parameters based on venous and arterial data were calculated. This was done for a range of $t_{½,k_{e0}}$ values: 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, and 240 minutes. In studies on respiration and analgesia a large variation in $t_{½,k_{e0}}$ values has been observed between 60 and 240 minutes.[69,68] From

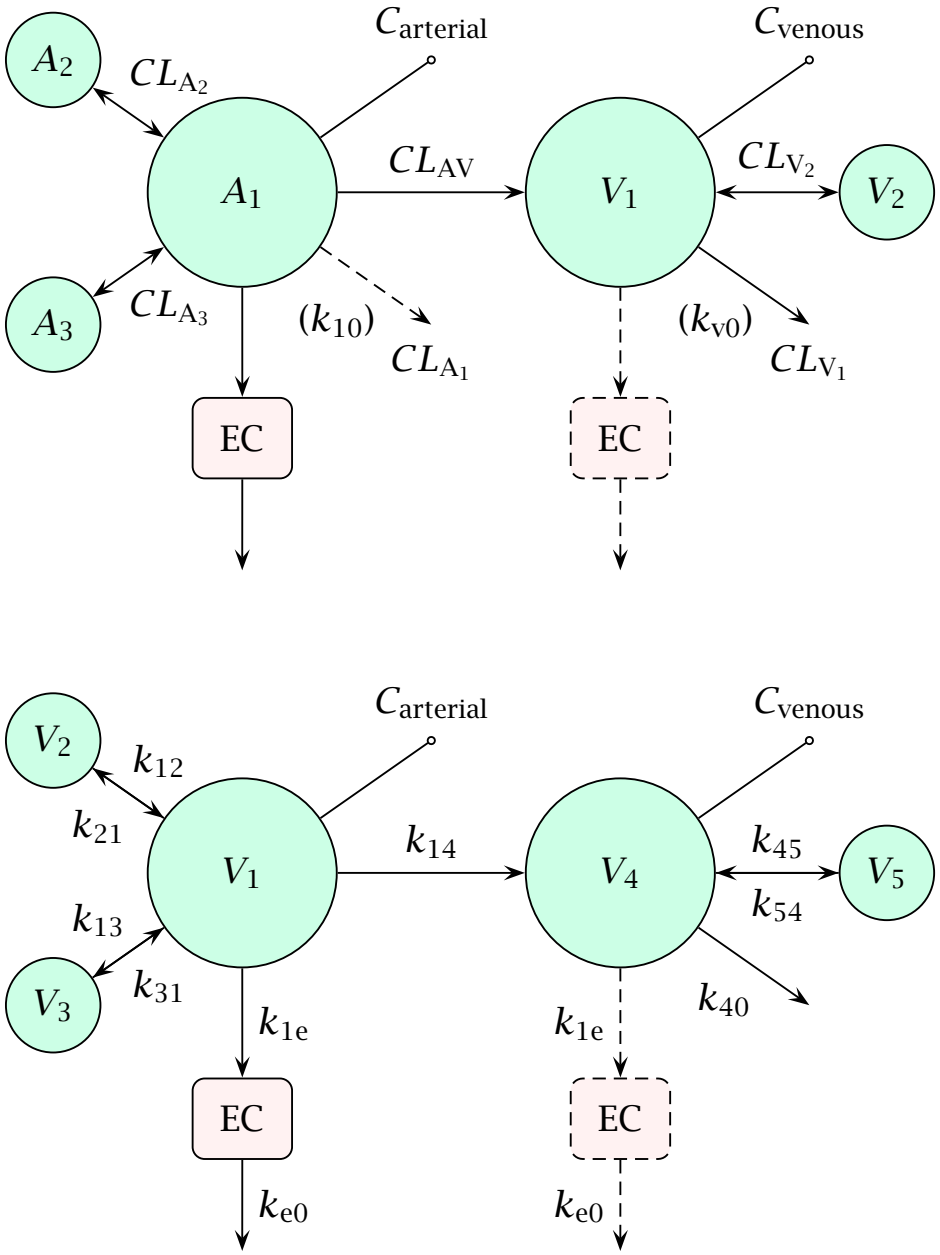

Figure 6.1: Two schematic representations of the pharmacokinetic (PK) model used to simultaneously analyze the arterial and venous morphine-6-glucuronide (M6G) data. **A**: Model in terms of volumes and clearances. A_1, A_2, and A_3 are arterial compartments, and V_1 and V_2 are venous compartments. CL_{A_1}, CL_{A_2}, CL_{A_3}, CL_{V_1}, and CL_{V_2}, are the clearances from compartments A_1, A_2, A_3, V_1, and V_2, respectively. An effect site has been postulated, driven by arterial M6G concentrations (EC linked to A_1) and an effect site driven by venous concentrations (EC linked to V_1; as if venous concentrations were equal to arterial ones). The latter representation is erroneous and tested in the current study. **B**: Model in terms of rate constants and NONMEM compartments. We assumed that k_{14} equals k_{10} (or CL_{A_1}/V_{A_1}). This has no influence on the model but indicated that V_4 and V_5 (and the clearance between them) have to be interpreted in relation to k_{14} (or CL_{AV}). The k_{e0} is the rate constant depicting the equilibration between blood and effect site. The k_{40} is the elimination rate from the central venous compartment and is equivalent to k_{v0}.

the 1000 simulation data sets, the median and 95% confidence intervals of the ratios of the parameters based on venous and arterial data were calculated. $t_{½,k_{e0}}$ values chosen below the values typically seen in respiratory and pain studies (1 hour) do reflect values that may occur when examining other end points, such as opioid-induced changes in electroencephalographic data.

Study II

To get an indication of the effect of using a biased PD parameter set, as may occur in clinical settings when PK and PD data sets derived from distinct arterial and venous concentration data sets are linked (*e.g.*, effect-controlled target-controlled infusion systems), we simulated the link between a PK model based on arterial data and a PD model based on venous data. Simulations were performed using the median estimates from Study I. To quantify the bias, we made a comparison to a simulation in which PK and PD models are both based on arterial data.

6.2 Results

All subjects completed the study without major side effects. Figure 6.2 shows the time course of the plasma concentrations of M6G for the samples obtained from arterial (radial artery) and venous (elbow) sites for 3 subjects. In Figure 6.3 the difference between arterial and venous M6G concentrations are plotted over time.

The data indicate that just after the 90-second infusion (with relatively high M6G concentrations), arterial concentrations exceeded the venous ones because the net drug flow is into the tissues of the arm. At later times (>40 minutes, with relatively low M6G concentrations), venous concentrations exceeded arterial concentrations because the net drug flow is from tissue to blood.

A schematic representation of the final "extended" PK model is given in Figure 6.1: for the arterial site (*i.e.*, body), 3 compartments were required (MOFV, 2 compartments = 2389.811 *versus* 3 compartments = 2167.616); for the venous site (*i.e.*, forearm), 2 compartments were required (MOFV 1 compartment = 4693.153 *versus* 2 compartments = 4418.097). Best, median, and worst data fits and goodness-of-fit plots are shown in Figure 6.2. Spaghetti plots (measured and predicted concentrations *versus* time) for the arterial and venous plasma concentration data are given in Figure 6.4. Without exception, the data were well described by the model. The model parameters are collected in Table 6.1. A significant equilibration delay was present between the central arterial and venous compartments ($t_{½,k_{v0}}$ = 2.00 ± 0.45 minutes, median ± SE). Figure 6.5 shows the results of simulation study I. For both values of γ, the results indicate that large biases are to be expected when using venous PK data as input to the PD model, albeit the magnitude of the bias depends on the "true" value for parameter $t_{½,k_{e0}}$ (for analgesia the true values range from 2 to 6 hours; for respiratory depression, from 1 to 2 hours). For parameter $t_{½,k_{e0}}$ the bias (as reflected by the ratio parameter derived from venous PK over arterial PK) is an underestimation of the "true" value ranging from a decrease of 60% at a "true" $t_{½,k_{e0}}$ value of 5 minutes to a decrease of 30% at a value of 240 minutes. For C50 the estimation from venous PK data yields a small bias (an overestimation ranging from +10% to -2%) in the $t_{½,k_{e0}}$ range of 5 to 90 minutes. At larger values of $t_{½,k_{e0}}$ the bias increases to an overestimation of 30% to 40% at $t_{½,k_{e0}}$ = 240 minutes. Parameter γ

Table 6.1: Pharmacokinetic Parameter Estimates

Parameter	Estimate	SE of estimate	ω^2	SE of ω^2
V_{A_1} (L)	4.45	0.69	0.07	0.04
V_{A_2} (L)	4.73	0.36	0.03	0.01
V_{A_3} (L)	5.07	0.27	0.02	0.01
CL_{A_1} (L/min)	0.14	0.006	0.03	0.01
CL_{A_2} (L/min)	0.55	0.10	-	
CL_{A_3} (L/min)	0.07	0.01	-	
$t_{½,k_{v0}}$ (min)	2.00	0.45	0.12	0.10
V_{V_1} (L)	0.05			
V_{V_2} (L)	1.88	0.38	0.68	0.25
CL_{V_2} (L/min)	0.06	0.01	0.27	0.18
σ^2 arterial	0.003	0.001		
σ^2 venous	0.02	0.006		

V_{A_1}, V_{A_2}, and V_{A_3} are the volumes of the arterial compartments A_1, A_2, and A_3, with intercompartmental clearances CL_{A_1}, CL_{A_2}, and CL_{A_3}, respectively.
$t_{½,k_{v0}}$ is the half-life of drug elimination from compartment V_1.
V_{V_1} is the volume of venous compartment V_1; V_{V_2} is the volume of venous compartment V_2 with intercompartmental clearance CL_{V_2}. V_{V_1} is derived from $V_{V_1} = CL_{A_1}/k_{v0}$,which implies that in the steady state, arterial and venous concentrations are equal.
The ω^2 are between-subjects variabilities (in the log-domain); the σ^2 are the residual errors.

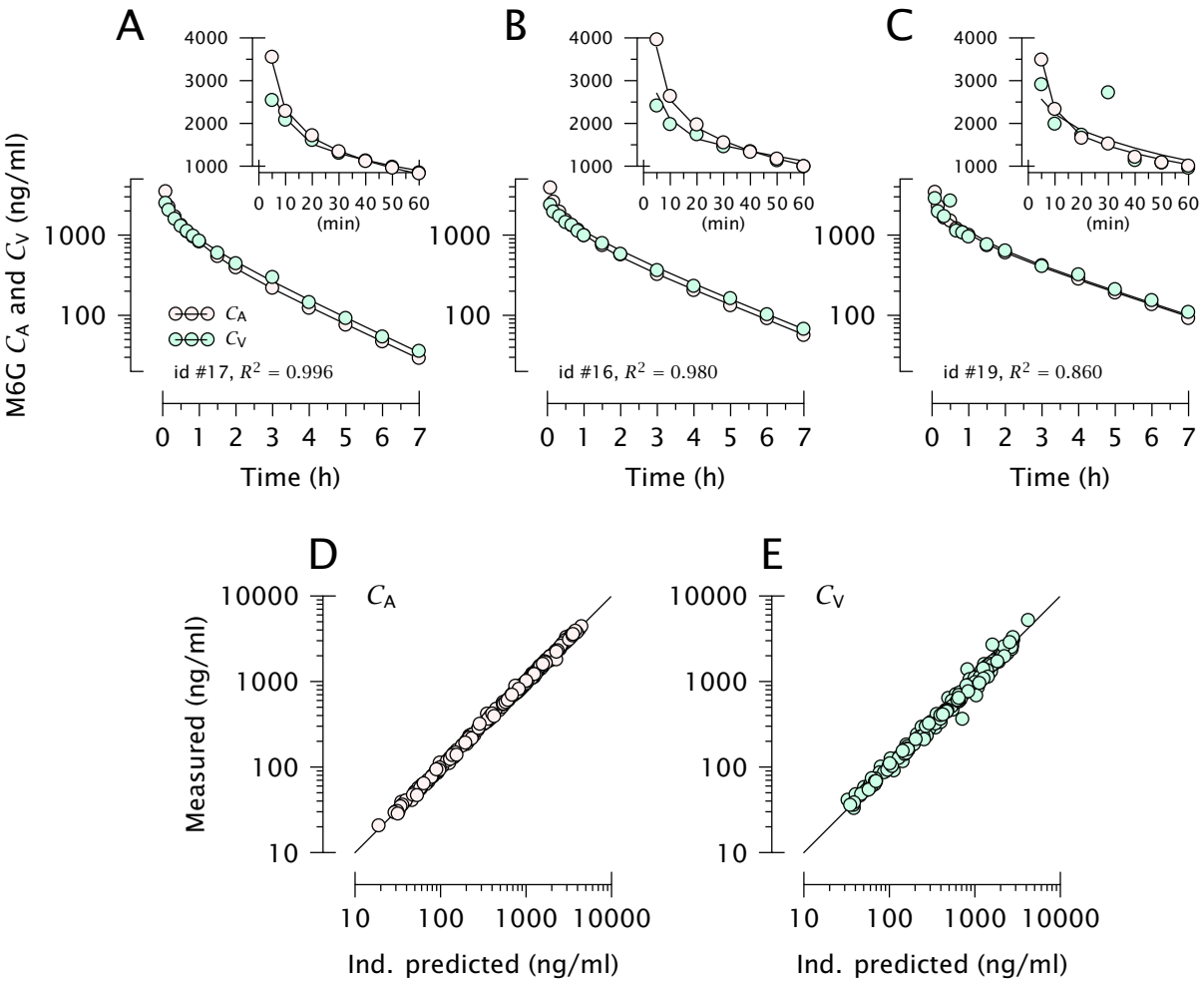

Figure 6.2: Best (**A**), median (**B**), and worst (**C**) pharmacokinetic (PK) data fits. Pink symbols, arterial samples; cyan symbols, venous samples. Solid lines, data fits. M6G, morphine-6-glucuronide. Inserts are the samples and data fits of the first hour of the experiment. **D** and **E**: Goodness-of-fit plots for the individual PK model for the arterial (**D**) and venous (**E**) concentrations. Shown are the observed (*y-axis*) *versus* individual predicted (*x-axis*) PK data.

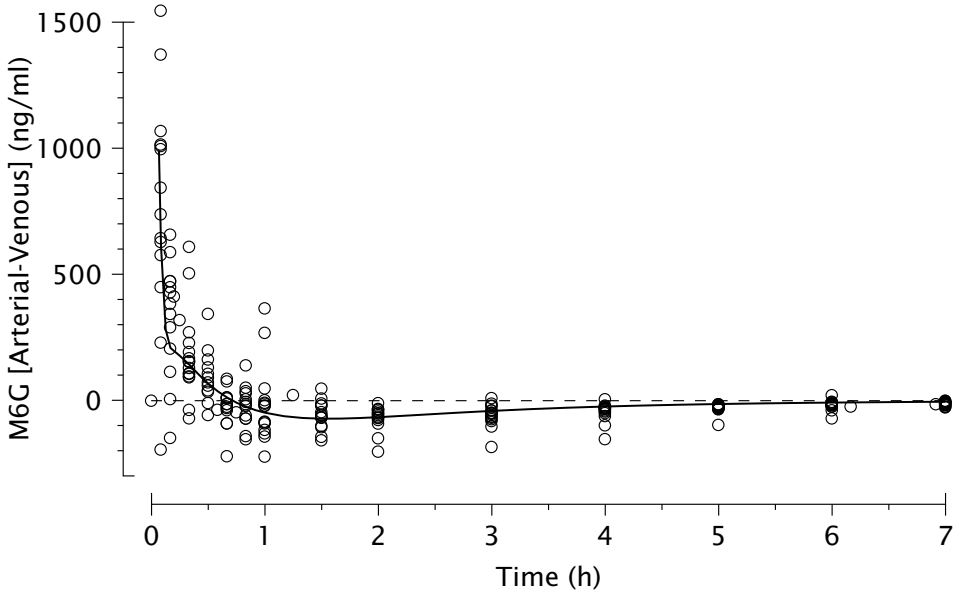

Figure 6.3: Difference between arterial and venous morphine-6-glucuronide (M6G) concentrations over time. The line through the data is the NONMEM population fit.

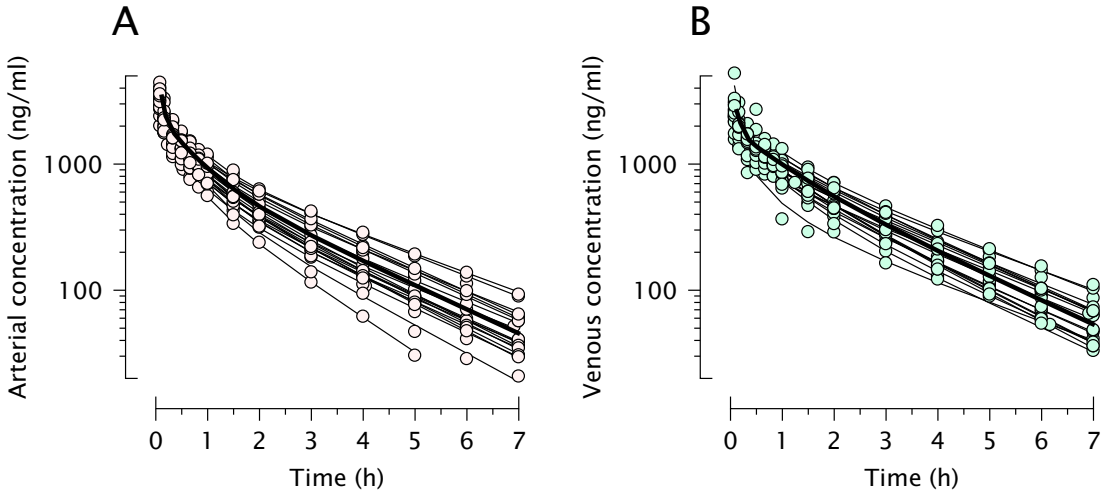

Figure 6.4: Spaghetti plots of measured and predicted morphine-6-glucuronide (M6G) concentrations in time. **A**: Measured (*solid circles*) and predicted (*solid black lines*) arterial concentrations. **B**: Measured (*solid circles*) and predicted (*solid black lines*) venous concentrations. The bold lines are the average model-based predicted concentrations.

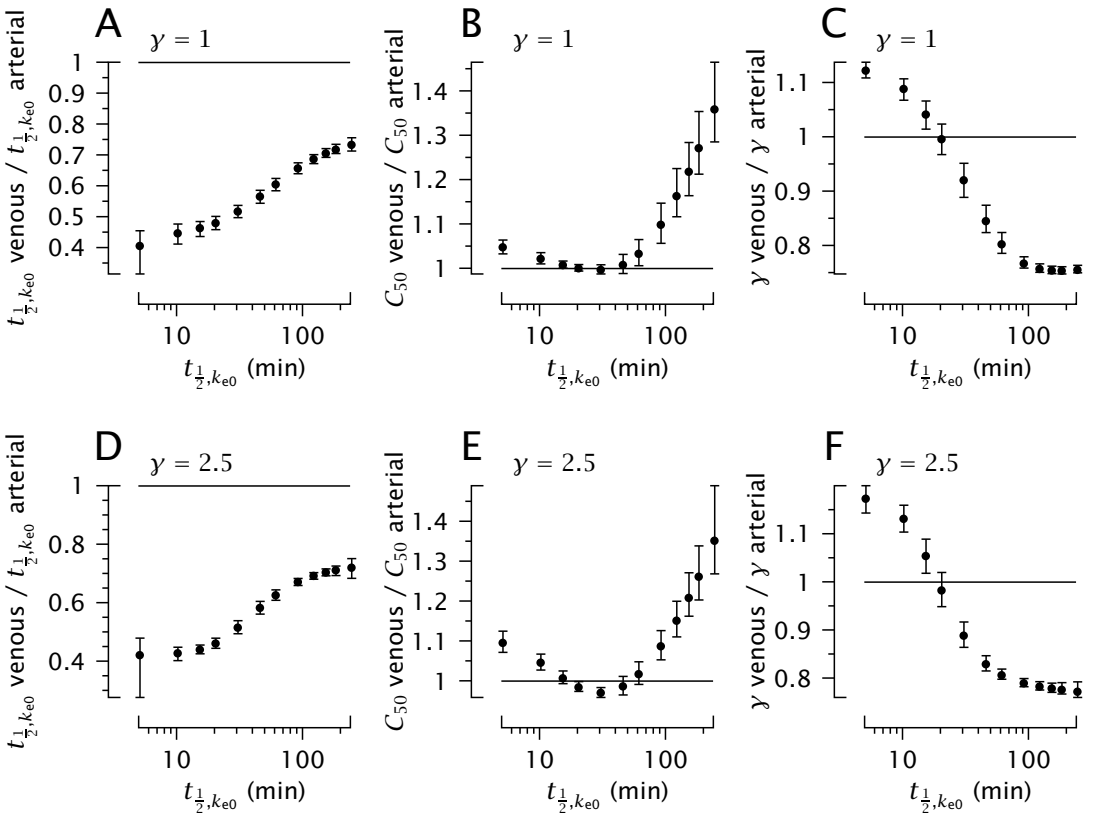

Figure 6.5: Results of simulation study I. **A** and **D**: Concentration site dependence on $t_{½,k_{e0}}$; **B** and **E**: Concentration site dependence on C_{50}; **C** and **F**: Concentration site dependence on γ. The ratio of the parameters obtained by using venous and arterial pharmacokinetic data are plotted against $t_{½,k_{e0}}$. A, B, and C are simulations with $\gamma = 1$; D, E, and F with $\gamma = 2.5$. $t_{½,k_{e0}}$ values range from 5 to 240 minutes. Values are median (of 1000 simulations) ± 95% confidence interval.

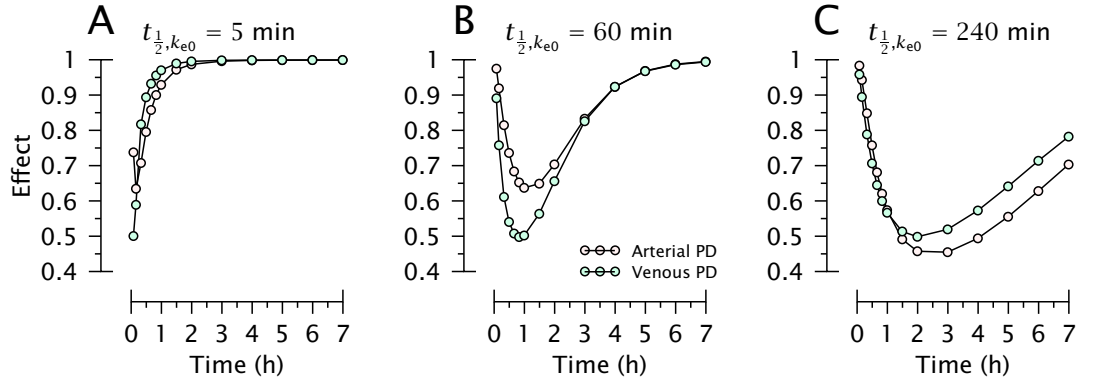

Figure 6.6: Results of simulation study II. Linking arterial pharmacokinetic (PK) data to pharmacodynamic (PD) models derived from venous (*cyan symbols*) and arterial (*pink symbols*) PK data. The data are simulated for 3 different values of $t_{½,k_{e0}}$: **A**: 5 minutes, **B**: 60 minutes, and **C**: 240 minutes. The effect simulated was a maximum of a 50% decrease in effect for the PD model derived from venous data.

is overestimated at $t_{½,k_{e0}}$ values <20 minutes by about 10%, whereas at larger values it is underestimated, reaching 25% at $t_{½,k_{e0}}$ = 240 minutes.

Figure 6.6 shows the results of simulation study II. The cyan symbols are the PD data that occur when an arterial PK set is linked to a PD parameter set derived from a venous PK data set for 3 values of $t_{½,k_{e0}}$ (5, 60, and 240 minutes). The pink symbols are the PD data derived from "arterial" PK and PD sets. The simulations were such that the maximum "venous" PD peak effect was 50% of control. As is obvious from the presented simulations, there are clear differences in effect between the arterial and venous PD models. For example, at low $t_{½,k_{e0}}$ values, venous peak effect exceeded arterial peak effect (for $t_{½,k_{e0}}$ = 60 minutes the venous peak effect is 30% greater at t = 60 minutes). However, at increasing values of $t_{½,k_{e0}}$ the differences decrease, and at $t_{½,k_{e0}}$ values between 150 and 180 minutes the arterial peak effect exceeds the venous peak effect (Figure 6.6C). For all values of $t_{½,k_{e0}}$, peak effect occurred somewhat earlier in the venous PD model.

6.3 Discussion

This study was designed to evaluate the effect of venous *versus* arterial blood sampling (derived from the cubital vein and radial artery, respectively) of the μ-opioid M6G on the bias of parameter estimates derived from PD models using simulated data of volunteers. We chose a combined arterial-venous model to model the AV differences in PK (which gives information of the kinetics at the site of sampling), rather than just a PK model that described the venous PK data in terms of an "arterial" model. The values of the arterial PK parameters were well in agreement with those presented in the literature.[68] Previous studies assessed the relevance of AV concentration differences on PK-PD modeling. For example, in the rat, Tuk *et al.*[93] measured arterial and venous concentrations of the benzodiazepine midazolam and linked them to effect (using electroencephalographic amplitude). Using a "traditional" effect-compartment model, differences in PD model parameters were apparent for EC_{50} (104 *versus* 86 ng/mL for arterial *versus* venous sampling) and k_{e0} (0.32 *versus* 313 min^{-1}). With an extended effect-compartment model (by characterizing the delay between arterial and venous sampling sites, comparable to our approach depicted in Figure 6.1), the model parameters did improve, although large differences did persist (EC_{50} = 89 ng/mL, k_{e0} = 2.5 min^{-1}).[93]

The AV concentration difference of a drug is, for a large part, determined by its interaction with the tissue at the venous sampling site (the venous concentration is not a reflection of the mixed venous/pulmonary artery concentrations). As was discussed by Gumbleton *et al.*,[37] mechanisms for the generation of AV concentration differences in the forearm arise from an elimination process (the drug is taken up by the muscles in the arm and metabolized in the muscle cells), by a distributional process (drug equilibration between plasma and tissue, which is dependent on the fraction of cardiac output going to the sample arm, perfusion and temperature-dependent capillary shunting, diffusion into and affinity for muscle tissue), or the combination of the two. M6G is a drug that is eliminated from the plasma exclusively through renal clearance. Because no tissue metabolic processes are known for M6G, the AV difference for this drug is determined solely by a distributional process in the forearm (equilibration between plasma and muscle tissue occurs at times >120 minutes, which is 4 times the half-life for the lower $t_{½,k_{e0}}$; see next paragraph). The PK-PD consequence of a large distributional AV concentration

difference is illustrated by Gumbleton *et al.*[37] showing that the determination of PD parameters will be highly biased when using a first-order effect-compartment model. In contrast, when the AV differences are related to an elimination process only (with instantaneous equilibration between plasma and tissue and a constant AV difference), little bias in parameter estimates is expected.[37]

Our results are in agreement with the theoretical studies of Tuk *et al.*[92] and Gumbleton *et al.*[37] However, our extended PK model differs significantly from their applied PK models. Most importantly, the venous part of our PK model has two compartments (V1 and V2), and theirs has only one. This makes comparison of our parameter k_{v0} not possible between studies. For the PK parameters of the venous compartments it can be calculated that clearance from the venous site is characterized by two half-lives, with values of 1.4 and 33 minutes, respectively. This suggests a secondary slow equilibration of M6G between arterial and venous blood and as such explains the large bias in the PD parameter estimates derived from venous blood samples. In our simulation study I we showed further that the bias in parameter estimates is critically dependent on the value of $t_{½,k_{e0}}$. When using venous M6G PK values as input to our first-order effect-compartment model and assuming that the "true" $t_{½,k_{e0}}$ value of M6G ranges from 60 to 240 minutes, as observed for M6G's effect on respiration and analgesia,[69,68] we would have underestimated $t_{½,k_{e0}}$ by 30% and γ by 25%, while the potency parameter (EC_{50}) would have been overestimated by about 40%. The estimations were also dependent on the value of the Hill coefficient γ (which may vary from 1 in respiratory studies to 2.5 in analgesia studies),[69,68] although this effect was less in magnitude (Figure 6.5).

In simulation study II, the effect of the use of biased PD parameters (*i.e.*, derived from venous blood samples) linked to an arterial PK set was explored. A situation in which the two are linked may occur in, for example, target-controlled infusion systems that incorporate PD model parameters to steer effect rather than target plasma concentration. The bias in predicted effect was dependent on the value of $t_{½,k_{e0}}$ with respect to the magnitude of effect and the timing of peak effect. The bias was such that a useful application of the model is not warranted in a clinical setting. Note, however, that if the PD model is derived from a venous PK set and linked to venous PK data, the bias would be minimal (but only when using an infusion scheme identical to that used in establishing the venous model), although the parameter estimates are biased in comparison with an arterial PK set and PD model.[92] Evidently, such an approach makes reliable comparisons with studies using arterial PK and PD models impossible and also is in violation of the principle that the effect site is directly linked to arterial rather than venous blood concentrations.

In conclusion, there are significant AV differences in M6G plasma concentration, related to a distributional process in the forearm. Biases exceeding 10% - 20% in PD model parameters will occur when linking venous concentration to effect, using the traditional effect-compartment model.

6.A Appendix: Linking Venous Compartments

It is assumed that the arterial morphine-6-glucuronide (M6G) concentrations are completely described by a 2- or 3-compartment model. The venous compartment then needs to be linked to the arterial pharmacokinetic (PK) model without affecting the latter, similar to linking an effect compartment.

For the amount of drug in the central (A_{V4}) and peripheral (A_{V5}) venous compartments we write (Figure 6.1B)

$$dA_{V4}/dt = k_{14} \cdot A_{V1} - k_{40} \cdot A_{V4} + k_{54} \cdot A_{V5} - k_{45} \cdot A_{V4} \quad (6.4)$$

$$dA_{V5}/dt = k_{45} \cdot A_{V4} - k_{54} \cdot A_{V5}, \quad (6.5)$$

where k_{14}, k_{54}, and k_{45} are rate constants between compartments V_1 and V_4, V_5 and V_4, and V_4 and V_5, respectively; k_{40} is the elimination rate constant from compartment V_4. We set k_{14} equal to k_{10} (= CL_{A_1}/V_{A_1}; Figure 6.1A) so that V_4 is not trivial in size but rather

$$V_4 = V_1 \cdot k_{14}/k_{40}. \quad (6.6)$$

This is an alternative yet exact method to keep the arterial PK part unaffected. We devised this method to avoid potential numerical problems in NONMEM with very small volume parameters. So k_{40} (Figure 6.1B) equals k_{v0} (= CL_{V_1}/V_{V_1}; Figure 6.1A); and $t_{½,k_{v0}}$ (= $\log(2)/k_{v0}$) is a parameter to be estimated.

It must be noted that, given the above, parameters V_{V_1}, V_{V_2}, and CL_{V_2} in Table 6.1 and Figure 6.1A are to be interpreted in relation to CL_{AV} (= CL_{A_1}), which will differ from the true arterial-venous clearance.