An Integrated Model for the Analysis of Pharmacokinetic Data from Microdialysis Experiments

Karin Tunblad,[1,2] Margareta Hammarlund-Udenaes,[1] and E. Niclas Jonsson[1]

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Purpose. To develop an integrated model for microdialysis data that incorporates all data including the recovery measurements in one model, and to compare this model to a previous model and the results from a noncompartmental analysis.

Methods. The models were developed in NONMEM. The modes of analysis were compared with respect to parameter estimates, model structures, gained mechanistic insight, and practical aspects.

Results. Both modeling approaches resulted in similar model structures. The parameter estimates in blood and brain from the models and the results from the noncompartmental analysis were comparable. Using the integrated model all data, that is, the total arterial concentrations, the venous and brain dialysate concentrations, and the recovery measurements, were analyzed simultaneously.

Conclusion. The theoretical benefits of the integrated model are related to the inclusion of the recovery in the model and the use of all collected data as it was observed. Thus, all data are described in a single model, corrections for the recovery and the protein binding are done within the model, and the dialysate observations are described by the integral over each collection interval. Thereby, the variability and the uncertainty in the model parameters are handled correctly to give more reliable parameter estimates.

KEY WORDS: Blood-brain barrier; microdialysis; modeling; morphine-6-glucuronide; M6G; noncompartmental analysis; NONMEM.

INTRODUCTION

Microdialysis is a quantitative method that measures the unbound concentration of a drug in a specific tissue. Therefore, the technique makes it possible to study the local pharmacokinetics of drugs, for example in the brain. To assess the transport across the blood-brain barrier (BBB) or other membranes, we need to address both the rate and the extent of transport. For this, continuous measurements of unbound drug concentrations in the brain and in the blood are crucial, which makes microdialysis a very useful method.

In short, one way of performing a microdialysis experiment to study the BBB transport of drugs is in the following way. Microdialysis probes, inserted into the brain tissue and in venous blood are perfused with artificial extracellular fluid (Ringer) at a low flow-rate, and fractions of dialysate are collected at certain intervals. The concentration in the dialysate gives a measure of the unbound tissue concentration. However, since there is a continuous flow through the microdialysis probe, the observed dialysate concentration will only be a fraction of the true unbound tissue concentration. This fraction is called the recovery or the extraction fraction (1). For quantitative measurements the recovery has to be determined *in vivo* in each microdialysis probe. In our studies we use the method of retrodialysis by drug to measure the recovery (2). This is accomplished by perfusing the probe with a Ringer solution containing a low concentration of the drug to be studied. The method assumes that the loss of drug from the perfusate to the surrounding tissue during retrodialysis is equal to the gain of drug from the tissue to the dialysate after systemic drug administration. After the calibration of the probes the perfusate is changed to a blank Ringer solution, and a wash-out period is allowed prior to drug administration.

Data from this type of experiments can be analyzed in a couple of different ways. One common approach is to use traditional noncompartmental analysis (NCA). This descriptive approach is fairly straightforward and gives individual estimates of some pharmacokinetic parameters, for example the clearance and the volume of distribution. However, there are limitations with this method that are related to the assumptions of error free recovery and protein binding measurements and the use of the mid time point as the time of the dialysate observation. In addition, in studies of the BBB transport this method only gives a measure of the extent, and not the rate of BBB transport.

Another approach is to build a mathematical model for the data. By nonlinear mixed effects modeling the population parameter estimates, the interindividual variability and the residual error can be assessed. In addition, in studies of the BBB transport both the rate and the extent of transport can be estimated. Another benefit is that the found model can be used for simulations. Simulations can be useful for study design optimization, for instance regarding the dosing regimen and the length of the collection intervals of the microdialysis samples.

In our studies the total arterial concentrations and the dialysate concentrations in brain and venous blood are measured. In the system we want to describe, the unbound arterial concentrations drive the unbound concentrations in the brain, and the unbound venous concentrations are in equilibration with the unbound arterial concentrations (Fig. 1). Devising a model for this system is technically challenging, since the concentrations that describe the drug distribution between the blood and the brain are not equal to the observed concentrations from the dialysates and from the regular blood sampling. Therefore certain assumptions regarding the recovery and the protein binding have been made in previous modeling approach (3). In that model the dialysate concentration measurements are corrected for the recovery prior to the data analysis, and the mid time point in each collection interval is used as the time of the observation. In addition, the arterial concentrations are corrected for the protein binding before the model development. Thereafter, in the estimation of the model parameters, the arterial data is used to assess the systemic pharmacokinetics, and the microdialysis data collected in the venous blood is used to obtain an estimate of the protein binding of the drug. This approach, which will be referred to as the restricted approach, has been used to model the local pharmacokinetics in the brain (see, e.g., Ref. 3). The ap-

[1] Division of Pharmacokinetics and Drug Therapy, Department of Pharmaceutical Biosciences, Uppsala University, SE-751 24 Uppsala, Sweden.

[2] To whom correspondence should be addressed. (e-mail: Karin.Tunblad@farmbio.uu.se)

ABBREVIATIONS: AUC, area under the concentration-time curve; BBB, blood-brain barrier; NCA, noncompartmental analysis; OFV, objective function value.

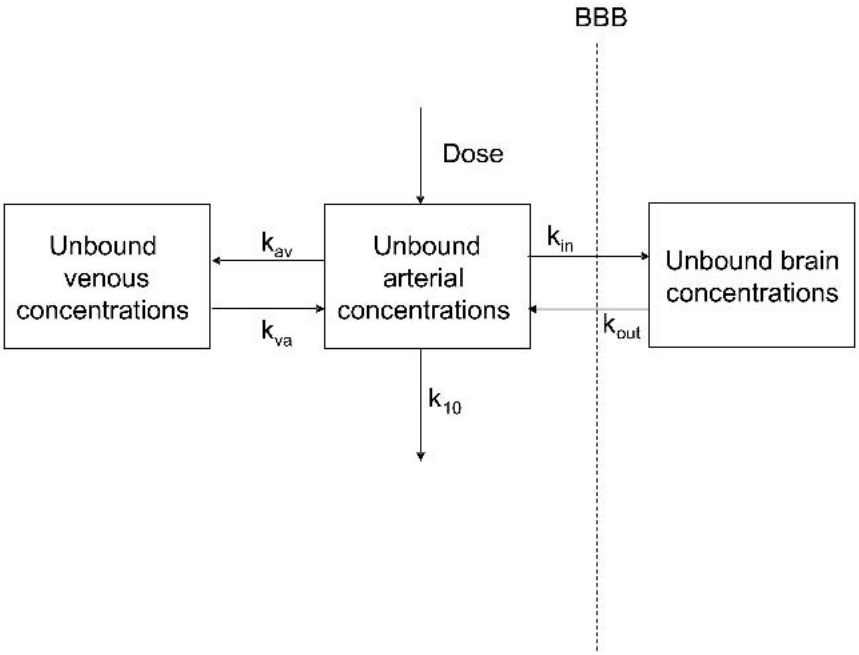

Fig. 1. The system describing the distribution of unbound drug between the blood and the brain compartments. The unbound arterial concentrations are in equilibrium with the unbound venous concentrations, and the unbound brain concentrations are governed by the unbound arterial concentrations. Abbreviations: BBB, blood-brain barrier; k_{av}, rate constant from the arterial to the venous blood; k_{va}, rate constant from the venous to the arterial blood; k_{10}, rate constant out of the blood; k_{in} rate constant from the blood into the brain; k_{out} rate constant from the brain to the blood.

proach is restricted in the sense that it assumes that there is no uncertainty in the recovery and in the protein binding, assumptions which are, of course, not true. In addition, by using the mid point in each collection interval as the time of the observation, linear pharmacokinetics and small changes in the concentrations over time in each collection interval is assumed (4,5). In the majority of situations it is unlikely that these assumptions will lead to qualitatively different conclusions. However, if the recovery is low, the protein binding of the drug is high, there is nonlinear pharmacokinetics or the half-life of the drug is short, i.e., changes in the concentrations over time is fast, these assumptions may influence the results.

The aim of the present analysis was to develop an integrated model for this type of microdialysis experiments. This model should include all the available observations, and corrections for the recovery and the protein binding should be done within the model. Thereby the variability and the uncertainty in the parameter estimates would be handled in a proper manner, giving the model better predictive properties. In addition, by modeling the observed dialysate concentrations, no assumptions regarding the time of the dialysate observation is made. To illustrate the integrated model, morphine data from a microdialysis experiment was used (3). The integrated model was compared to the restricted model and to the results from an NCA data analysis strategy.

MATERIALS AND METHODS

The Data

The data used comes from a 2-day study in rats where the objective was to investigate if co-administration of probenecid would affect the BBB transport of morphine (3). In the present study we used the data from the first experimental day (n = 8) when morphine alone was administered. In short, one microdialysis probe (CMA/20, CMA, Stockholm, Sweden) was implanted into the jugular vein, and one probe (CMA/10) was inserted into striatum in the brain. A cannula was inserted into the femoral artery for regular blood sampling, and the drug was infused via a catheter in the femoral vein. To obtain the recovery value for each probe the probes were calibrated using the method of retrodialysis by drug prior to drug administration (2). During this period the probes were perfused with Ringer containing morphine at a low concentration (100 ng/ml), and fractions of dialysate were collected. The retrodialysis period yielded approximately four samples per probe. After a wash-out period, morphine was administered as a 4-h exponential infusion. Fractions of dialysate were collected in 10–15 min intervals, and arterial samples were drawn at predefined points in time during the infusion and two hours post infusion. The morphine data used in the estimation of the pharmacokinetic parameters were the total arterial concentrations, the dialysate concentrations in brain and venous blood and the retrodialysis measurements.

The Noncompartmental Data Analysis

The average recovery value, calculated from the retrodialysis data, was determined for each probe according to:

$$\text{Recovery}_{in\ vivo} = \frac{\sum_{i=1}^{x} \frac{C_{in} - C_{out,i}}{C_{in}}}{x} \quad (1)$$

C_{in} is the morphine concentration entering the probe, $C_{out,i}$ is the *i*th observed morphine concentration leaving the probe, and x is the number of recovery observations in each probe. The dialysate concentrations were corrected for the average individual recovery to obtain estimates of the unbound tissue concentration. The unbound systemic pharmacokinetic parameters were calculated from the arterial data after correction for the individual value of the protein binding. This was obtained by comparing the area under the concentration-time curve (AUC) in the venous blood (unbound concentrations) to that in the arterial blood (total concentrations). The ratio between the unbound steady state concentration in the brain to that in the venous blood ($C_{u,ss,brain}/C_{u,ss,blood}$) was used to determine the equilibration ratio across the BBB, which represents the extent of BBB equilibration.

Model-Based Data Analysis

General Modeling Specifications

Nonlinear mixed effects modeling in the computer program NONMEM version VIß (6) was used for the data analysis. The final run was confirmed using NONMEM version V (6). The first order conditional estimation method with interaction (FOCE INTER) was used for all analysis. Model selection was based on the objective function value (OFV) from the NONMEM output and graphical analysis using Xpose version 3.1 (7). A drop in the OFV of 3.84 between two nested models corresponds to $p < 0.05$, which was regarded as being statistically significant.

To describe the data, models comprising one or several compartments in the brain and in the blood were considered. An exponential model was used to describe the interindividual variability, that is, the difference between the individual parameter estimate and the typical value of the parameter (Eq. 2).

$$P_i = P \cdot \exp^{\eta i} \tag{2}$$

P_i denotes the *i*:th individual's parameter value, P the typical value of the parameter and η_i the individual random effect that accounts for the difference between the typical parameter and the individual estimate. To describe the residual error, that is, the difference between the observed and the predicted concentration, the additive, the proportional and the slope-intercept models were considered (6). The derived parameters from the models were the unbound parameters.

The Restricted Model

Morphine recovery for each probe was first calculated from the retrodialysis data according to Eq. 1. The arterial concentrations were modeled after correction for the individual protein binding, which was obtained from the NCA. Thus, the systemic pharmacokinetic parameters were obtained from the arterial data. The unbound population parameters from the analysis of the arterial data were used as fixed values in the analysis of the brain concentrations, while retaining the observed data in the dataset (8). The mid point in time in the collection interval was taken as the time of the dialysate observation.

The rate of BBB transport was parameterized in terms of clearance into the brain (CL_{in}) and clearance out of the brain (CL_{out}). The unbound volume of distribution in the brain ($V_{u,brain}$) was used as a fixed value (3) and calculated according to:

$$V_{u,brain} = \frac{A_{br} - V_{bl} \cdot C_{bl}}{C_{u,br}} \tag{3}$$

A_{br} represent the total amount of drug in the brain, V_{bl} is the volume of blood in the brain, C_{bl} is the total drug concentration in the blood and $C_{u,br}$ is the unbound brain concentration of the drug. The volume of blood in the rat brain has been estimated as 14 μl (9). Since the amount of morphine in the brain is small, mass transfer from the brain back to the blood was neglected.

The Integrated Model

In this approach, the systemic pharmacokinetics was described using all the data collected in blood; i.e., the total arterial concentrations, the recovery measurements in venous blood and the venous dialysate concentrations. The models considered either assumed the same pharmacokinetics, or allowed for a delay in the distribution between the arterial and the venous blood. The transport across the BBB was parameterized as CL_{in} and CL_{out}. To describe the brain parameters, models with or without mass transfer from the brain back to the blood were tried. The microdialysis data, collected as fractions, was modeled using an output compartment (6), similar to what would be used for urine collection data. The dialysate concentrations were predicted from the model by integrating the concentration-time profile in each dialysate collection interval. The overall aim was to estimate the systemic pharmacokinetic parameters, the brain parameters, the recovery and the protein binding simultaneously.

RESULTS

The Restricted Model

Using the restricted approach a two-compartment model was identifiable both in the blood and in the brain (Fig. 2). The following set of differential equations was used to describe the morphine distribution in the blood (Eqs. 4 and 5) and in the brain (Eqs. 6 and 7).

$$\frac{dA_1}{dt} = R_{exp} + k_{21} \cdot A_2 - (k_{12} + k_{10}) \cdot A_1 \tag{4}$$

$$\frac{dA_2}{dt} = k_{12} \cdot A_1 - k_{21} \cdot A_2 \tag{5}$$

$$\frac{dA_3}{dt} = k_{in} \cdot A_1 + k_{43} \cdot A_4 - (k_{out} + k_{34}) \cdot A_3 \tag{6}$$

$$\frac{dA_4}{dt} = k_{34} \cdot A_3 - k_{43} \cdot A_4 \tag{7}$$

R_{exp} represents the rate of the exponential infusion of morphine. A_1, A_2, A_3, and A_4 are the amounts of drug in the central blood compartment, the peripheral blood compartment, the central brain compartment, and the peripheral brain compartment, respectively. The rate constants k_{12}, k_{21}, k_{34}, and k_{43} represent the rate constants within the brain and the blood compartments, k_{10}, the rate constant out of the blood, k_{in} the rate constant from the blood to the brain and

k_{out} the rate constant out of the brain. Using these equations the unbound parameters were derived. The parameters describing the rate of the BBB transport, that is, CL_{in} and CL_{out}, were calculated according to:

$$CL_{in} = k_{in} \cdot V_1 \tag{8}$$

$$CL_{out} = k_{out} \cdot V_{u,brain,1} \tag{9}$$

where V_1 and $V_{u,brain,1}$ are the unbound volumes of distribution in the central blood and brain compartments, respectively.

The unbound arterial concentrations and the unbound brain concentrations were predicted according to Eqs. 10 and 11, where y_{ij} corresponds to the *j*th observation in the *i*th individual. Slope-intercept models best described the residual error in both the blood and the brain. The proportional errors are represented by ε_1 and ε_3, and ε_2 and ε_4 represent the additive errors.

$$y_{ij} = \frac{A_1}{V_1} \cdot (1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij} \tag{10}$$

$$y_{ij} = \frac{A_3}{V_{u,brain,1}} \cdot (1 + \varepsilon_{3,ij}) + \varepsilon_{4,ij} \tag{11}$$

The Integrated Model

Using the integrated model the systemic parameters, the blood probe recovery and the brain parameters, including the brain probe recovery, were estimated all together. However, during the development of this model the typical values of the recovery of the blood and brain probes were used as fixed values to adjust the dialysate concentrations. Fixing the recovery simplified the model building and decreased the run times for the data analysis. An example of a model file and the corresponding dataset for the integrated model is included in the Appendix.

The systemic pharmacokinetics of morphine was best described by a two-compartment model with no delay in the distribution between the arterial and the venous blood compartments (Fig. 3, Eqs. 12–14). In the brain a one-compartment model with bi-directional transport of morphine across the BBB sufficiently described the data (Fig. 3, Eq. 15).

$$\frac{dA_1}{dt} = R_{exp} + k_{va} \cdot A_2 + k_{41} \cdot A_4 + k_{out} \cdot A_5 - (k_{14} + k_{av} + k_{in} + k_{10}) \cdot A_1 \tag{12}$$

$$\frac{dA_2}{dt} = k_{av} \cdot A_1 - k_{va} \cdot A_2 \tag{13}$$

$$\frac{dA_4}{dt} = k_{14} \cdot A_1 - k_{41} \cdot A_4 \tag{14}$$

$$\frac{dA_5}{dt} = k_{in} \cdot A_1 - k_{out} \cdot A_5 \tag{15}$$

R_{exp} represents the rate of the exponential infusion of morphine. A_1, A_2, A_4 and A_5 correspond to the amount of morphine in the arterial, venous, peripheral and the brain compartment, respectively. The rate constants k_{14} and k_{41} represent the rate constants between the arterial and the peripheral compartments, k_{av} and k_{va} the rate constants between the

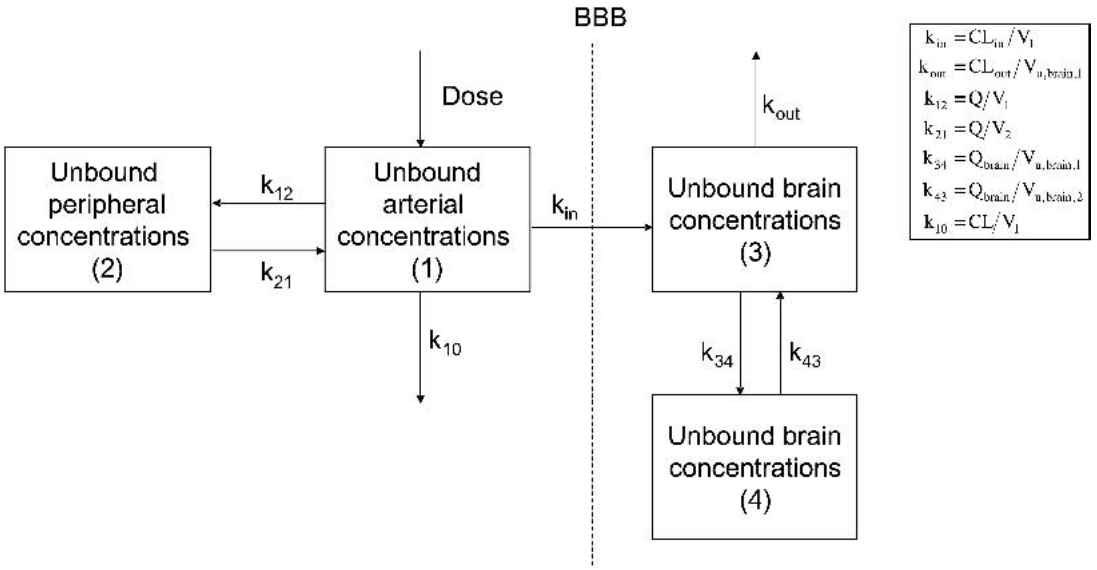

Fig. 2. The restricted model for the distribution of morphine in the blood and in the brain. The arterial concentrations were corrected for the protein binding prior to the model development. Thereafter, the unbound arterial morphine concentrations were modeled, and the unbound brain concentrations, corrected for the brain probe recovery, were subsequently conditioned on the arterial unbound population parameter estimates. The compartment numbers used in Eqs. 4–7 are given within parentheses. The conversion from the rate constants to the estimated parameters is given in the legend. Abbreviations: BBB, blood-brain barrier; k_{12} and k_{21}, rate constants between the central and the peripheral compartments; k_{34} and k_{43}, rate constants between the brain compartments; k_{10}, rate constant out of the blood; k_{in} rate constant from the blood into the brain; k_{out} rate constant out of the brain; CL_{in}, influx clearance; CL_{out}, efflux clearance; $V_{u,brain,1}$, central volume of distribution in the brain; $V_{u.brain,2}$, peripheral volume of distribution in the brain; Q_{brain}, intercompartmental clearance in the brain; CL, clearance; Q, intercompartmental clearance; V_1, the central volume of distribution and V_2, the peripheral volume of distribution.

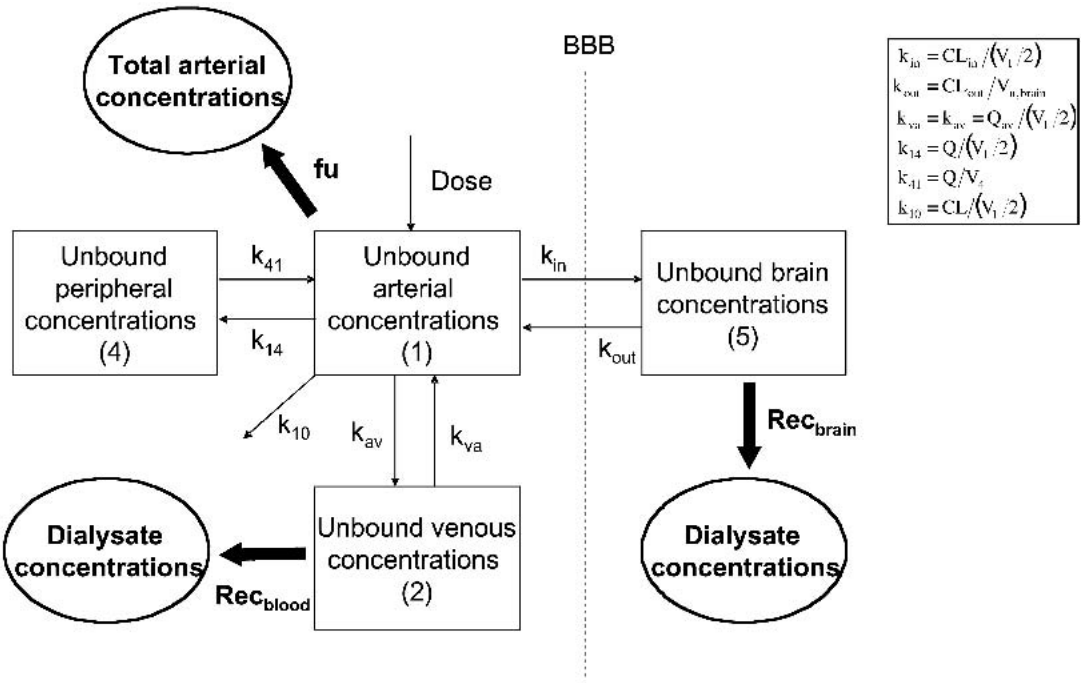

Fig. 3. The integrated model describing the systemic pharmacokinetics and the blood-brain barrier transport of morphine. The elliptical boxes represent the observed data, and the thick arrows show the corrections that are made to obtain the true concentrations that describe the distribution of unbound drug between the blood and the brain. The thin arrows represent mass transport. Corrections for the recovery and the protein binding were done within the model. The compartment numbers used in Eqs. 12–15 and 18–19 are given within parentheses. The conversion from the rate constants to the estimated parameters is given in the legend. Abbreviations: BBB, blood-brain barrier; Rec_{blood}, blood probe recovery; Rec_{brain}, brain probe recovery; f_u, unbound fraction; k_{av}, rate constant from arterial to venous blood; k_{va}, rate constant from venous to arterial blood; k_{10}, rate constant out of the blood; k_{in} rate constant from the blood into the brain; k_{out} rate constant from the brain to the blood; k_{12} and k_{21}, rate constants between the arterial and the peripheral blood compartments; CL_{in}, influx clearance; CL_{out}, efflux clearance; $V_{u,brain}$, unbound volume of distribution in the brain; CL, clearance; Q_{av}, intercompartmental clearance in the blood; Q, intercompartmental clearance and V_1 and V_4 the central and the peripheral volume of distribution, respectively .

arterial and the venous compartments, k_{10}, the rate constant from the blood, k_{in} the rate constant from the blood to the brain and k_{out} the rate constant from the brain back to the blood. Similarly to the restricted model the BBB transport was parameterized in terms of CL_{in} and CL_{out} and, therefore, an estimate of $V_{u,brain}$ was required. In the model $V_{u,brain}$ was used as a fixed parameter, and CL_{in} and CL_{out} were calculated from:

$$CL_{in} = k_{in} \cdot (V_1/2) \tag{16}$$

$$CL_{out} = k_{out} \cdot V_{u,brain} \tag{17}$$

The dialysate concentrations in the venous blood and in the brain were expressed as the integral over each collection interval, represented by t_1 and t_2 (Eqs. 18 and 19). These equations are equivalent to DADT (3) shown in the model file in the Appendix.

$$C_{dialysate,blood} = \int_{t_1}^{t_2} \frac{A_2}{(V_1/2)} \cdot Recovery_{blood}\, dt \tag{18}$$

$$C_{dialysate,brain} = \int_{t_1}^{t_2} \frac{A_5}{V_{u,brain}} \cdot Recovery_{brain}\, dt \tag{19}$$

In the model, the central volume of distribution (V_1) was equally divided between the two blood compartments, and mass transfer from the tissues into the dialysates was neglected. The dependent variables predicted in the integrated model were the same as the observed concentrations; that is, the total arterial concentrations and the integrated venous and brain dialysate concentrations. The predictions were obtained according to:

$$y_{ij} = \left(\frac{A_1}{V_1/2}\right) \Big/ fu \cdot (1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij} \tag{20}$$

$$y_{ij} = \frac{C_{dialysate,blood}}{TIN} \cdot (1 + \varepsilon_{3,ij}) + \varepsilon_{4,ij} \tag{21}$$

$$y_{ij} = \frac{C_{dialysate,brain}}{TIN} \cdot (1 + \varepsilon_{5,ij}) + \varepsilon_{6,ij} \tag{22}$$

TIN represents the length of the collection interval for the dialysates and f_u denotes the fraction unbound. Figure 4 shows the observed data, the population predictions and the individual predictions of the arterial concentrations and the dialysate concentrations obtained from the integrated model. The residual error for the blood probe recovery was best described using a proportional error model, and for the brain probe recovery an additive error model was sufficient.

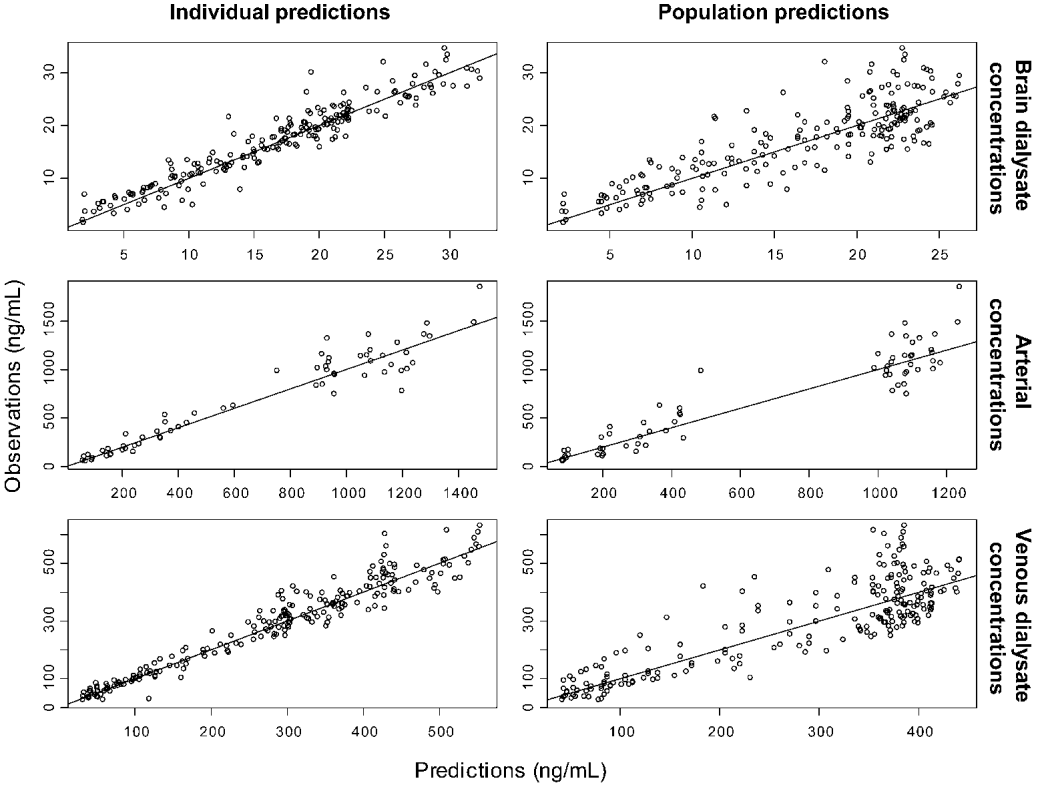

Fig. 4. The observed data and population predictions and individual predictions of the brain dialysate concentrations, the venous dialysate concentrations, and the total arterial concentrations obtained from the integrated model.

Comparing the Results from the Different Data Analysis Strategies

Both modeling approaches resulted in similar structural models, and the systemic pharmacokinetic parameter estimates from the two models were in agreement with the results from the NCA (Table I).

The extent of BBB equilibration, represented by $C_{u,ss,brain}/C_{u,ss,blood}$ and the ratio between CL_{in} and CL_{out}, were similar for both the modeling and the NCA approaches

Table I. The Unbound Systemic Parameters of Morphine Calculated from the Noncompartmental Analysis, and the Unbound Population Parameter Estimates from the Two Models Expressed as Typical Values (Relative Standard Errors [RSE %])

		Calculated values and population estimates (RSE %)				
	Parameter	Noncompartmental analysis	Restricted model	IIV (%)	Integrated model	IIV (%)
PK parameters	CL (ml/min)	26.7 (5.4)	27.5 (5.9)	16 (16)	26.6 (6)	14 (31)
	V1 (ml)	—	445 (18)	NE	339 (22)	23 (48)
	V2 (ml)	—	515 (11)	NE	553 (17)	NE
	Vss (ml)	901 (10)	960‡	—	892‡	—
	fu (%)	77.4 (5.3)	—§	—	77.6 (5.3)	NE
	Recovery (%)	48.4 (4.6)	—§	—	45.9 (4.2)	14 (55)
Residual error	Arterial blood* (%)	—	17.7 (8.1)		16.9 (18)	
	Arterial blood† (ng/ml)	—	13.8 (50)		33.2 (45)	
	Venous dialysate* (%)	—	—		12.3 (5.7)	
	Venous dialysate† (ng/ml)	—	—		14.7 (20)	
	Recovery* (%)	—	—		10.7 (19)	

CL, clearance; IIV, interindividual variability; V1 and V2, volume of distribution in compartment one and two, respectively; Vss, volume of distribution at steady state; fu, fraction unbound; NE, not estimated.

* Proportional error.

† Additive error.

‡ The sum of V1 and V2.

§ Calculated values from the noncompartmental analysis were used.

Table II. The Calculated Values and the Population Parameter Estimates of the Blood-Brain Barrier Transport of Morphine from the Noncompartmental Analysis and the Two Models Expressed as Typical Values (Relative Standard Errors [RSE %])

		Calculated values and population estimates (RSE %)				
	Parameter	Noncompartmental analysis	Restricted model	IIV (%)	Integrated model	IIV (%)
PK parameters	CL_{in} (μl/min)	—	19.6 (8.1)	22 (43)	19.6 (7.2)	20 (54)
	CL_{out} (μl/min)	—	71 (7.6)	NE	74.4 (6.9)	NE
	CL_{in}/CL_{out}	—	0.276	—	0.263	—
	$C_{u,ss,brain}/C_{u,ss,blood}$	0.29	—	—	—	—
	Recovery (%)	10.5 (5.4)	—‡	—	10.4 (5.5)	12 (66)
Residual error	Brain* (%)	—	11.8 (17)		—	
	Brain† (ng/ml)	—	12.4 (31)		—	
	Brain dialysate* (%)	—	—		6.48 (47)	
	Brain dialysate† (ng/ml)	—	—		2.04 (23)	
	Recovery* (%)	—	—		0.92 (18)	

CL_{in}, clearance into the brain; IIV, interindividual variability; CL_{out} clearance out of the brain; $C_{u,ss,brain}/C_{u,ss,blood}$, the ratio of unbound brain to blood concentrations at steady state; CL_{in}/CL_{out}, the ratio of clearance into and out of the brain and NE, not estimated.
* Proportional error.
† Additive error.
‡ Calculated values from the noncompartmental analysis were used.

(Table II). From the two models the values of the rates of the BBB transport, that is, CL_{in} and CL_{out}, were comparable (Table II). The calculated blood probe recovery was 48.4%, which was similar to the estimated value of 45.9% from the integrated model. The fraction unbound was calculated to be 77% and estimated as 78% (Table I).

DISCUSSION

In this paper, a new modeling approach to handle the data from microdialysis experiments is presented. In addition, data analysis using noncompartmental methodology is compared to a model based approach. A summary of the characteristics using each approach is presented in Table III.

General Aspects of the Different Data Analysis Strategies

The NCA is easy and straightforward to apply to this type of data. However the analysis can only be used for descriptive purposes. By fitting a model to the data the model can be used for simulations, which can be useful in the optimization of the design of a study. For example, it would be possible to simulate the concentration-time profiles for differently sized collection intervals, and to select the more optimal sampling times depending on the pharmacokinetic properties of the drug to be studied.

Both models successfully described the data. However the integrated model has both theoretical and practical advantages. The theoretical benefits are related to the incorporation of the recovery in the model, and the use of all collected data as it was observed. Thus, all data are described with just a single model and corrections for the recovery and the protein binding are done within the model. Thereby the variability and the uncertainty in the model parameters are handled in a proper manner, giving more reliable parameter estimates. By estimating the recovery both the uncertainty in the recovery value *per se*, the inter probe variability and the inter occasion variability can be assessed. This is important if the precision in the recovery estimate is low, as the tissue concentrations will have an uncertainty that is neglected if the dialysate

Table III. Benefits and Drawbacks of Using the Noncompartmental Methodology, the Restricted Model, and the Integrated Model for the Analysis of Microdialysis Data, with the Emphasis on the Brain Drug Delivery

Characteristics	Noncompartmental analysis	Restricted model	Integrated model
Data transformation required prior to the data analysis	Yes	Yes	No
Assumptions required about the time of the observation of the microdialysis data	Yes	Yes	No
Uncertainty and variability in the measurement of the recovery is recognized	No	No	Yes
Uncertainty in the measurement of the protein binding is recognized	NA	No	Yes
All blood data are used to estimate the systemic pharmacokinetics	No	No	Yes
Possibility to investigate time delays in drug distribution between arterial and venous blood	No	No	Yes
Level of complexity in the data analysis	Low	Medium	High
Computer capacity required	Low	Medium	High
The extent of blood-brain barrier equilibration can be estimated	Yes	Yes	Yes
The rates of blood-brain barrier transport can be estimated	No	Yes	Yes
Possibility to estimate the volume of distribution in the brain	No	No	Yes
Usefulness for simulations	NA	Medium	High

NA, not applicable.

concentrations are simply conditioned on the average recovery value, as is done using the restricted model and the NCA.

In this study the recovery was estimated using the method of retrodialysis by drug (2), that is, the recovery was estimated prior to drug administration. This technique assumes that the recovery is constant during the experiment following the retrodialysis period. An alternative to this method would be to use an internal standard, ideally the drug under investigation. Thereby an estimate of the recovery would be obtained for each microdialysis fraction collected during the experiment. For example time dependence of the recovery of tritiated water was demonstrated in blood but not in the brain by Sjöberg and co-workers (10). However, Bouw *et al.,* showed that it is sufficient to estimate the recovery for morphine prior to the systemic drug administration (2). Other examples are zidovudine (11) and codeine (12), where the recovery estimated prior to drug administration was similar to the recovery estimated from simultaneous retrodialysis during the experiments. Nevertheless it would be an advantage to get an estimate of the recovery from each microdialysis sample due to possible changes in the recovery over time. In the present study, it was assumed that the recovery was constant during the pharmacokinetic experiment using both the restricted and the integrated model.

Using the integrated model the venous concentration measurements are both used to assess the protein binding and to stabilize the model in blood. With the restricted model, the arterial measurements, adjusted for the protein binding, are used to estimate the systemic pharmacokinetics. Consequently, the majority of the observations in blood are not used to directly assess the systemic pharmacokinetics. This can be a drawback when experiments are performed over several days, since the regular blood sampling sometimes fails due to clotting of the catheters. Thereby some animals will lack information from the arterial blood. Since the brain concentrations are conditioned on the unbound arterial parameter estimates with the restricted model, the brain concentrations in these individuals will have to be conditioned on the typical parameter estimates in blood. In contrast, with the integrated model the venous data would be used to assess the individual blood parameter estimates if information from the arterial blood is lacking. However, as the typical parameter estimates in both the brain and the blood were similar using either modeling approach it seems, at least in this example, sufficient to only use the arterial data to assess the systemic pharmacokinetics as well as the BBB transport. In addition, as the ratio of CL_{in}/CL_{out} from both modeling approaches and the $C_{u,ss,brain}/C_{u,ss,blood}$ ratio from the NCA were similar, it seems sufficient to use only the venous data to assess the extent of the BBB transport.

The mid point in the collection interval is generally taken as the time of the observation for the microdialysis samples. This is sufficient if the collection intervals are short in relation to the half-life of the drug, or if the changes in concentrations over time are slow (4,5). If, however, the fluctuations in the concentrations are rapid or the sampling intervals are long relative to the half-life of the drug, the use of the mid point in time may introduce an error in the estimation of the pharmacokinetic parameters. In the integrated model each observation is described by the integral of the concentration-time profile in the corresponding collection interval (i.e., AUC). Thus no assumptions are made regarding the changes in the dialysate concentration within a collection interval. However, it was assumed that there was no mass transfer from the tissues into the dialysates. This is reasonable since the amount of drug recovered in the microdialysis samples is very small.

From a practical point of view it is more straightforward and less error prone to construct the dataset for the integrated than the restricted model, since no observations are adjusted prior to the construction of the dataset.

Structural Models

A two-compartment model using either model described the pharmacokinetics in the blood. By incorporating both the arterial and the venous data in the integrated model it was possible to investigate if there was a delay in the drug distribution between the two blood compartments. This could not be considered in the restricted model or in the NCA. For morphine no such delay could be justified. With the integrated model, a one-compartment model described the brain data, while a two-compartment model could be justified using the restricted model. This discrepancy can be explained by the uncertainty in the protein binding and the recovery being neglected in the restricted model. Thereby the restricted model is in general likely to detect a more complex model.

Considerations for the Transport Across the BBB

By fitting a model to the data both the rate of BBB transport (CL_{in} and CL_{out}) and the extent of equilibration across the BBB (CL_{in}/CL_{out}) can be assessed. In contrast, only the extent ($C_{u,ss,brain}/C_{u,ss,blood}$) of equilibration can be calculated using the NCA. Since the rate of transport may vary for different drugs even though the extent of equilibration is similar (3,13,14), it is necessary to model the data to investigate both phenomena.

The integrated model allows for mass transfer in both directions across the BBB, while the restricted model does not. Under the assumption that morphine is not totally metabolized in the brain (15) it is more mechanistically correct to include mass transfer from the brain back to the blood. Allowing the BBB transport to be bi-directional, both the plasma and the brain data can be analyzed simultaneously since it is then not necessary to condition the brain concentrations on the blood parameters. Nevertheless, the parameter estimates describing the BBB transport of morphine were similar for both modeling approaches, confirming that the mass transfer from the brain back to the blood is of less importance for morphine from a quantitative perspective.

The restricted model requires a value of the volume of distribution in the brain, while the integrated model does not necessarily need this. However, since the amount of drug entering the brain is small it will have a low impact on the systemic pharmacokinetics, thereby making the volume of distribution in the brain difficult to estimate. Although it was possible to estimate this parameter for morphine, the parameter estimate was highly sensitive to the initial estimate of the parameter. Consequently, to estimate both the CL_{in} and the CL_{out} a good notion of the value of the volume of distribution in the brain should be known.

In conclusion, the theoretical benefits of the integrated model are related to the incorporation of the recovery in the model, and the use of all collected data as it was observed.

APPENDIX #1

An Example of the NONMEM Code Used in the Integrated Model

```
$PROBLEM Morphine

$INPUT ID TIME AMT RATE DV EVID CMT CIN FLAG TIN

$DATA intervaldayl.dta

$SUBROUTINE ADVAN6 TRANS1 TOL=3

$MODEL C
          COMP = ARTERIAL
          COMP = VENOUS
          COMP = OUTPUT
          COMP = PERIPHERAL

$PK
     REC  = THETA (1) *EXP (ETA (1))          ; Recovery
     CUT  = CIN– (CIN*REC)                    ; Dialysate concentration, recovery data
     CL   = THETA (2) *EXP (ETA (2))          ; Clearance
     V1   = THETA (3) *EXP (ETA (3))          ; Central volume of distribution
     Q    = THETA (4)                         ; Intercompartmental CL
     V2   = THETA (5)                         ; Peripheral volume of distribution
     FU   = THETA (6)                         ; Fraction unbound
     QAV  = THETA (7)                         ; Arterial-venous intercompartmental CL
     VA   = V1/2                              ; Arterial V1
     VV   = V1/2                              ; Venous V1
     K    = CL/VA
     K12  = QAV/VA
     K21  = QAV/VV
     K14  = Q/VA
     K41  = Q/V2

$DES      DADT(1)  = – (K12+K14+K)*A(1)+K21*A(2)+K41*A(4)
          DADT(2)  = K12*A(1) –K21*A(2)
          CP       = A(2)/VV
          DADT(3)  = CP*REC
          DADT(4)  = K14*A(1) –K41*A(4)

$ERROR  A1 = A(1)
        A3 = A(3)

         IF (FLAG.EQ.1) THEN                  ; Venous data
          IPRED = A(3)/TIN
          W     = SQRT (IPRED*THETA (8)) **2+THETA(11)**2)
          RESV  = EPS(1)
         ENDIF

         IF (FLAG.EQ.2) THEN                  ; Recovery data
          RESV  = EPS(2)
          IPRED = CUT
          W     = THETA(9)*IPRED
         ENDIF

         IF (FLAG.EQ.3) THEN                  ; Arterial data
          IPRED = (A(1)/VA)/FU
          W     = SQRT((IPRED*THETA(10))**2+THETA(12)**2)
          RESV  = EPS(3)
         ENDIF

         IRES  = DV-IPRED
         IWRES = IRES/W
         IF (W.EQ.0) W=1
         Y=IPRED+W*RESV
```

APPENDIX #2

An Extract from the Dataset Used in the Integrated Model

ID	TIME	AMT	RATE	DV	EVID	CMT	CIN	FLAG	TIN
1	0	30	1	-	1	1	-	0	-
1	0	-	-	-	2	3	0	0	10
1	0.01	-	-	20.35	0	1	41.8	2	-
1	0.02	-	-	23.60	0	1	41.8	2	-
1	0.03	-	-	26.58	0	1	41.8	2	-
1	0.04	-	-	24.75	0	1	41.8	2	-
1	10	-	-	23.21	0	3	0	1	10
1	10	-	-	-	2	–3	0	0	10
1	10	-	-	-	2	3	0	0	10
1	20	-	-	129.79	0	3	0	1	10
1	20	-	-	-	2	–3	0	0	10
1	20	-	-	-	2	3	0	0	10
1	30	0	-	187.89	0	3	0	1	15
1	30	-	-	1147.60	0	1	-	3	-
1	30	-	-	-	2	–3	0	0	15
1	30	-	-	-	2	3	0	0	15

Abbreviations: AMT, amount; RATE, rate of drug input; DV, dependent variable; EVID, event identification (0 = observation record, 1 = dose event, and 2 = turn on/switch off the output compartment); CMT, compartment (1 = arterial, 3 = turn on/observation in the output compartment, and –3 = switch off the output compartment); CIN, perfusate concentration for the recovery measurements; FLAG (0 = dose event/turn on the output compartment, 1 = dialysate observation, 2 = recovery observation, and 3 = arterial observation); TIN, the time of the dialysate collection interval.

Thus, all data are described with just a single model and corrections for the recovery and the protein binding are done within the model. Thereby, the variability and the uncertainty in the model parameters are handled correctly, to give more reliable estimates of the pharmacokinetic parameters. In addition, this approach allows for study design optimization through simulations.

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REFERENCES

1. P. M. Bungay, P. F. Morrison, and R. L. Dedrick. Steady-state theory for quantitative microdialysis of solutes and water in vivo and in vitro. *Life Sci.* **46**:105–119 (1990).
2. M. R. Bouw and M. Hammarlund-Udenaes. Methodological aspects of the use of a calibrator in in vivo microdialysis-further development of the retrodialysis method. *Pharm. Res.* **15**:1673–1679 (1998).
3. K. Tunblad, E. N. Jonsson, and M. Hammarlund-Udenaes. Morphine blood-brain barrier transport is influenced by probenecid co-administration. *Pharm. Res.* **20**:618–623 (2003).
4. L. Ståhle. Pharmacokinetic estimations from microdialysis data. *Eur. J. Clin. Pharmacol.* **43**:289–294 (1992).
5. P. N. Patsalos, W. T. Abed, M. S. Alavijeh, and M. T. O'Connell. The use of microdialysis for the study of drug kinetics: some methodological considerations illustrated with antipyrine in rat frontal cortex. *Br. J. Pharmacol.* **115**:503–509 (1995).
6. S. L. Beal and L. S. Sheiner. NONMEM user's guide, *NONMEM Project Group.* University of California at San Fransisco, 1994.
7. E. N. Jonsson and M. O. Karlsson. Xpose–an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput. Methods Programs Biomed.* **58**:51–64 (1999).
8. J. Wade and M. O. Karlsson. PAGE meeting. Available at www.page-meeting.org/ (1999).
9. U. Bickel, O. P. Schumacher, Y. S. Kang, and K. Voigt. Poor permeability of morphine 3-glucuronide and morphine 6-glucuronide through the blood-brain barrier in the rat. *J. Pharmacol. Exp. Ther.* **278**:107–113 (1996).
10. P. Sjöberg, I.-M. Olofsson, and T. Lundqvist. Validation of different microdialysis methods for the determination of unbound steady-state concentrations of theophylline in blood and brain tissue. *Pharm. Res.* **9**:1592–1598 (1992).
11. Y. Wang, S. L. Wong, and R. J. Sawchuk. Microdialysis calibration using retrodialysis and zero-net flux: application to a study of the distribution of zidovudine to rabbit cerebrospinal fluid and thalamus. *Pharm. Res.* **10**:1411–1419 (1993).
12. R. Xie and M. Hammarlund-Udenaes. Blood-brain barrier equilibration of codeine in rats studied with microdialysis. *Pharm. Res.* **15**:570–575 (1998).
13. M. R. Bouw, R. Xie, K. Tunblad, and M. Hammarlund-Udenaes. Blood-brain barrier transport and brain distribution of morphine-6-glucuronide in relation to the antinociceptive effect in rats–pharmacokinetic/pharmacodynamic modelling. *Br. J. Pharmacol.* **134**:1796–1804 (2001).
14. M. Hammarlund-Udenaes. The use of microdialysis in CNS drug delivery studies. Pharmacokinetic perspectives and results with analgesics and antiepileptics. *Adv. Drug Deliv. Rev.* **45**:283–294 (2000).
15. J. F. Ghersi-Egea, B. Leninger-Muller, G. Suleman, G. Siest, and A. Minn. Localization of drug-metabolizing enzyme activities to blood-brain interfaces and circumventricular organs. *J. Neurochem.* **62**:1089–1096 (1994).