Journal of Pharmacokinetics and Pharmacodynamics, Vol. 33, No. 5, October 2006 (© 2006)
DOI: 10.1007/s10928-006-9023-3

Diazepam Pharamacokinetics from Preclinical to Phase I Using a Bayesian Population Physiologically Based Pharmacokinetic Model with Informative Prior Distributions in Winbugs

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Received April 15, 2005—Final May 25, 2006—Published Online June 29, 2006

Modelling is an important applied tool in drug discovery and development for the prediction and interpretation of drug pharmacokinetics. Preclinical information is used to decide whether a compound will be taken forwards and its pharmacokinetics investigated in human. After proceeding to human little to no use is made of these often very rich data. We suggest a method where the preclinical data are integrated into a whole body physiologically based pharmacokinetic (WBPBPK) model and this model is then used for estimating population PK parameters in human. This approach offers a continuous flow of information from preclinical to clinical studies without the need for different models or model reduction. Additionally, predictions are based upon single parameter values, but making realistic predictions involves incorporating the various sources of variability and uncertainty. Currently, WBPBPK modelling is undertaken as a two-stage process: (i) *estimation (optimisation) of drug-dependent parameters by either least squares regression or maximum likelihood and* (ii) *accounting for the existing parameter variability and uncertainty by stochastic simulation. To address these issues a general Bayesian approach using WinBUGS for estimation of drug-dependent parameters in WBPBPK models is described. Initially applied to data in rat, this approach is further adopted for extrapolation to human, which allows retention of some parameters and updating others with the available human data. While the issues surrounding the incorporation of uncertainty and variability within prediction have been explored within WBPBPK modeling methodology they have equal application to other areas of pharmacokinetics, as well as to pharmacodynamics.*

KEY WORDS: bayesian analysis; population pharmacokinetics; physiologically based pharmacokinetic models; rat; human; diazepam.

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1567-567X/06/1000-0571/0 © 2006 Springer Science+Business Media, Inc.

GLOSSARY

WBPBPK model	Whole body physiologically based pharmacokinetic model,
MCMC	Monte Carlo Markov Chain,
MVN_p	p-dimensional multivariate normal distribution,
IG	inverse gamma distribution,
IW	inverse Wishart distribution,
K_p	tissue-to-plasma partition coefficient,
K_{p_b}	tissue-to-blood partition coefficient,
R	blood-to-plasma partition coefficient,
fu	fraction of drug unbound in plasma,
fu_b	fraction of drug unbound in blood,
CL_{INT}	hepatic intrinsic clearance,
$p(A \mid B)$	conditional probability distribution of A given B,
y_{kij}	either concentrations or log-concentrations depending on whether, normality or log-normality is the more appropriate assumption of the data,
$f_k(\cdot)$	structural model,
Z_i	$p \times q$ covariate-effect design matrix for individual i,
μ	vector of q fixed effects parameters,
$\Omega(p \times p)$	inter-individual variance-covariance matrix,
σ_k^2	residual error variance for the kth response,
trace plot	line connecting successive samples plotted against iteration number; useful graphics for investigating convergence,
(Kernel) density plot	a refinement of a histogram or frequency plot. The variable is plotted on the x-axis and the frequency is plotted on the y-axis. Kernel density plot is computed based on the Fast Fourier Transform (FFT) algorithm as suggested by Silverman, *Applied Statistics, Royal Statistical Society*, **33** (1982).
LI	liver,
KI	kidney,
BR	brain,
SPL	intestine,
ST	stomach,
MU	muscle from the hind limb,
AD	adipose,
SK	skin after removal of hair,
TE	testes,
HT	heart,
LU	lungs,
ART	arterial blood pool,
VEN	mixed venous blood pool.

INTRODUCTION

Whole body physiologically based (WBPBPK) models predict drug kinetics using knowledge about an organism's anatomy and physiology, aided by drug specific data. Compared, for example, to empirical modelling, which uses a sum of exponentials, which is purely descriptive of the observed behaviour of the compound, WBPBPK modelling provides a mechanistic and more realistic description of the behaviour of the drug in various tissues and blood. It allows the prediction of the pharmacokinetic behaviour of a compound only from its descriptors, such as lipophilicity, molecular size and shape. Although not currently realizable, such information combined with a generic WBPBPK model may enable a reasonable initial prediction of the likely behaviour *in vivo* in some tissues, especially those involving passive diffusion processes. These *in silico* computations may be combined with *in vitro* data, such as plasma protein binding, microsomal or hepatocyte intrinsic clearance and cell membrane permeability, which would allow for the inclusion of active processes involved in metabolism and membrane transport. Corroboration and further refinement of this generic physiological model can only be performed in animals, although the main objective is to predict likely human pharmacokinetics. Traditionally, the allometric approach, which assumes that species differences are driven by body size alone, has been the method used for inter-species scaling. However, this approach is limited as it does not account for species differences in active processes. WBPBPK modelling offers an alternative method for inter-species scaling to help decide on the first dose in human. Human studies are initially conducted in healthy subjects. Body weight and composition, hepatic and renal functions vary among patients. Patients also differ in the severity of the disease, consumption of other drugs, etc. WBPBPK modelling offers an improvement upon conventional approaches by providing a mechanistic framework for exploring the impact of these components and their variability on the pharmacokinetic profile in any tissue.

The current approach to WBPBPK modelling, termed naïve pooled data analysis, as the name suggests, pools animal tissue data (data usually comes from a small number of animals per time point), implicitly assuming the data come from a single animal, which is clearly erroneous. The main difficulty in estimation with WBPBPK models is the large number of parameters, often comparable to the number of observations in a typical PK experiment. It is desirable to estimate the distribution of individual characteristics and the associated population quantities. Ideally, we would also like to determine how these quantities vary as functions of individual characteristics, such as sex, age and body mass. The goal is then to estimate the distribution of population characteristics rather than a single set

of parameters representing the "average subject". Yet the majority of the reported studies in the literature use WBPBPK models to make single subject predictions by assuming only a structural model (1–3). Another issue is that physiologically based pharmacokinetics is currently a two-stage process where first the drug-dependent parameters (tissue-to-plasma concentration ratios, clearances, fraction unbound in plasma, blood-to-plasma ratio) are estimated, followed by acknowledging the variability in those parameters by performing stochastic simulations. Due to the difficulty of parameter estimation, it is also common practice to reduce the number of parameters to be estimated in a pharmacokinetic model. This is done by either fixing all but a few parameters to assumed values or by setting up a model with very few parameters, such as a one- or two-compartment model. The first approach has the serious problem of producing inaccurate estimates and underestimating uncertainty when the parameters to be fixed are not known accurately (4). The second approach has been used successfully (5) but has the limitation of not allowing realistic multi-compartment models to be fit. Recent developments in statistics and specifically Bayesian analysis using Monte Carlo Markov Chains (MCMC), have made possible parameter estimation of physiological models with formal inclusion of prior knowledge.

Whole body physiologically based models are occasionally used for inter-species extrapolation. However, while highly informative the current practice after having proceeded to Phase I human studies (healthy volunteers) is to abandon much of the preclinical data, including the model itself. This is because human data are invariably limited to plasma, blood and urine. Phase I and later phases often involve fitting compartment models to interpret data, which although having the potential to be somewhat more mechanistic than empirical models (e.g., sum of exponentials) are still not fully mechanistic. Due to the large number of parameters in WBPBPK models and the inability of parameter estimation in a frequentist framework, these models are not used for studying human pharmacokinetics. There arises the question as to whether to retain, collapse or abandon the animal WBPBPK model. Although this poses a very important problem with significant consequences, it has not been fully investigated. Previously, we proposed a methodology based on global sensitivity analysis for formally collapsing the structural WBPBPK model (6). Here, we explore retaining the full mechanistic animal physiological model for predicting human drug disposition using a population Bayesian approach. This approach allows retention of the mechanistic WBPBPK model with updating some PK parameters using the available human data.

Bayesian analysis is a method that produces statistical distributions (called 'posterior distributions') of the parameter values rather than single

point estimates. These posterior distributions are consistent with both the experimental data and the prior assumptions. The posterior distributions can be approximated by random draws using MCMC simulations. If a population model (7) is used, the Bayesian approach yields posterior distributions not only for the parameters of each subject but also for the population. This approach has been applied successfully to classical PK compartment models (5, 8) and more recently proposed in a WBPBPK context for toxicological experiments with repeated measurements with human data (e.g., 9–11).

The current work is focused on WBPBPK modelling of animal data and its subsequent extrapolation to predict human pharmacokinetics, which were addressed by using a population approach in a Bayesian framework. We illustrate the application to the pharmacokinetics of diazepam in rats and human, the problem that motivated this work.

METHODOLOGICAL CONSIDERATIONS

General Concepts in Bayesian Population WBPBPK Models

Hierarchical Models

Here, we describe a typical Bayesian population WBPBPK model. Our notation is generally based for consistency on that used by (12), where the concepts for Bayesian analysis for population PK/PD models are developed. Our work is an extension of their approach to WBPBPK models. Suppose, we have a number (n_i) of PK measurements for M responses made on each of L individuals, who are indexed by j. Denote the jth measurement of the kth response for individual i by y_{kij} and the associated time by t_{kij}. Further, denote the p-dimensional vector of PK parameters for individual i by θ_i and the residual error variance for the kth response by σ_k^2. At the first of the three stages in our hierarchical model, we typically assume

$$p\left(y_{kij}\left|\theta_i, \sigma_k^2\right.\right) \propto N\left(f_k\left(\theta_i;\, t_{kij};\, D_i\right),\, \sigma_k^2\right),$$
$$i = 1, \ldots, L, \quad j = 1, \ldots, n_i, \quad k = 1, \ldots, M,$$

where throughout, $p(A\,|B)$ denotes the conditional probability distribution of A given B. Here the y_{kij} are either concentrations or log-concentrations depending on whether normality or log-normality is the more appropriate assumption of the data. The structural model $f_k(\cdot)$, which is a function of individual specific parameters θ_i, time t_{kij} and the ith individual dosing history D_i, is defined on the same scale.

At the second stage of the model, we make distributional assumptions regarding the individual-specific PK parameter vectors θ_i:

$$p(\theta_i | \mu, \Omega) = \mathrm{MVN}_p(Z_i, \mu, \Omega), \quad i = 1, \ldots, L,$$

where $\mathrm{MVN}_p(\cdot, \cdot)$ denotes a p-dimensional multivariate normal distribution, Z_i is a $p \times q$ covariate-effect design matrix for individual i, μ is a vector of q fixed effects parameters and $\Omega(p \times p)$ is the inter-individual variance–covariance matrix.

The third stage of the hierarchical model comprises the prior specification, in which prior distributions are assigned to σ^2, μ and Ω:

$$p\left(\sigma^2\right) = \mathrm{IG}(a, b), \quad p(\mu) = \mathrm{MVN}_p(\eta, H), \quad p(\Omega) = \mathrm{IW}(R, \rho),$$

where $\mathrm{IG}(\cdot, \cdot)$ and $\mathrm{IW}(\cdot, \cdot)$ denote inverse-gamma and inverse-Wishart distributions, respectively. The values of a, b, η, H, R, ρ, also called *hyperparameters*, must be stated explicitly.

A schematic representation of a general population WBPBPK model in Bayesian framework is given in Fig. 1.

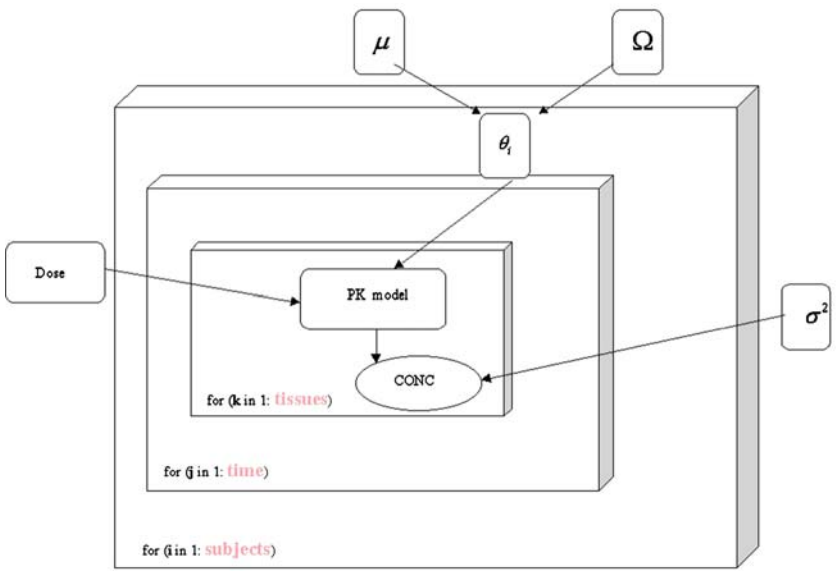

Fig. 1. Schematic representation of a population WBPBPK model (see text for definitions).

Priors

Traditionally, the distributional form of the priors has been chosen for mathematical convenience so that closed form posteriors may be derived analytically. For instance, an inverse-gamma prior, which is the conjugate prior for the normal variance, is typically specified for σ_k^2. Nowadays with the availability of several reliable methods for sampling from non-standard distributions the choice of priors is no longer restricted. In our studies non-informative priors for the intra-individual variance σ_k^2 and informative and vague priors for Ω have been used.

Convergence

Two issues arise when considering convergence: (i) is the chain stationary, i.e., at what point does the chain forget where it started and (ii) how many samples must be generated to make accurate predictions. A number of formal techniques exist for addressing these issues and many of them have been implemented in the CODA software package (13). It is informative to inspect the "trace" plot, where a line connecting successive samples is plotted against iteration number.

Software

WinBUGS is a general software package for performing Bayesian inference. However, until recently it could not handle WBPBPK models as it lacked a routine for simultaneous solution of ordinary differential equations. Recently, such a routine, in particular a Runge–Kutta fourth order procedure, was added to WinBUGS. Alternatively, another software package specifically written for Bayesian inference for WBPBPK models is MCSim, authored by Gelman *et al*. could be used (9). We choose WinBUGS, which as general software for Bayesian inference offers greater flexibility and reliability.

Diazepam WBPBPK Model in the Rat

The parameters of any WBPBPK model can be classified as drug-dependent (tissue-to-plasma partition coefficients *Kps*, fraction unbound in plasma fu, blood-to-plasma ratio R, intrinsic clearances CL_{INT}, etc.) and drug-independent (blood flows, tissue volumes, etc.). The experimental data together with the WBPBPK structural model are described in detail in (14). Here only a brief description is provided and the system of ordinary differential equations describing tissue disposition is given in Appendix. Tissue and arterial blood concentration-time profiles following an intravenous infusion over 5 min, were collected following administration of

1 mg/kg diazepam to each of 24 male Sprague-Dawley rats. Four of these animals were then sacrificed at each of the following times 7, 10, 20, 35, 95 and 245 min from the start of the infusion when various tissues were dissected out for analysis. These tissues were liver (LI), kidney (KI), brain (BR), intestine (SPL), stomach (ST), muscle from the hind limb (MU), adipose (AD), skin after removal of hair (SK), testes (TE), heart (HT) and lungs (LU). Plasma samples were taken from the carotid artery at those same times, as well as at 2 and 5 min into the infusion, and plasma obtained by centrifugation. Drug was administered by constant-rate infusion into the jugular vein (mixed venous compartment). The hepatic compartment receives drug input directly from the hepatic artery as well as from the splanchnic organs via the hepatic portal vein. The lungs close the circulation loop and receive blood at a flow rate equal to the cardiac output. Elimination is assumed to occur entirely from the liver compartment, as extrahepatic metabolism and renal excretion of diazepam are minor. The whole body PBPK model for diazepam (Fig. 2) comprises 12 tissue compartments and two blood compartments (ART—arterial and VEN—mixed venous). Each tissue is assumed to have perfusion-rate limited distribution and be represented by a single, well-stirred compartment, with the extent of drug distribution being characterized by the equilibrium tissue-to-venous blood concentration ratio, Kp_b ($Kp_b = Kp \times R$).

Organ and tissue volumes as well as blood flows for a 250 g standard weight rat (SWR) are given in Table I. Tissue weights were taken from the literature (15). Blood flows, assumed unaffected by drug administration,

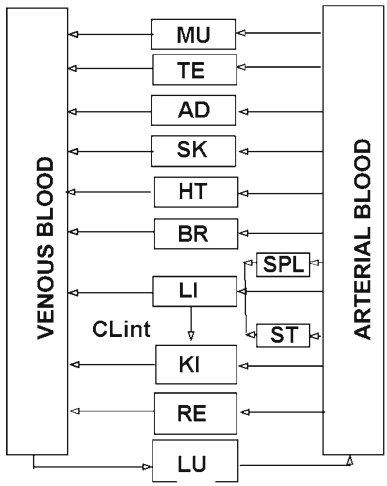

Fig. 2. Diazepam WBPBPK model. See text for definition of symbols.

Table I. Physiological Parameter Values for Rat (15, 16) and Human (17)

	Rat		Human	
Compartment	Blood flow (mL/min)	Weight (g)	Blood flow (L/h)	Weight (kg)
Whole Body[a]	80	250	360	70.0
VEN	80	13.6	360	3.9
ART	80	6.8	360	1.7
LU	80	1.2	360	0.47
LI[b]	3.55	11	23.4	1.8
KI	16.61	2	68.4	0.31
ST	1.9	1.1	3.60	0.15
GI	20.25	15	50.4	1.01
MU	16.25	125	61.2	30
AD	2.55	10	18.0	12.5
SK	7.1	43.8	18.0	2.6
BR	0.78	1.2	43.2	1.4
HT	4.2	1	14.4	0.33
TE	1.9	2.5	3.6	0.035
Rest of body	4.91	15.8	55.8	16.8

[a] Cardiac output and total body weight.
[b] Autonomous liver flow via the hepatic artery. Total liver flow is the sum of the flows to liver, stomach and intestine.

were reported for 300g rats (16). We calculated the fraction of different blood flows to the cardiac output for 300 g rats and used the same fraction to compute the blood flows for 250 g rats. The fraction unbound in plasma (f_u) was experimentally determined to be 0.15 and the blood-to-plasma concentration ratio (R) was assumed to be 1 (14).

In our study the drug-dependent parameters (Kps and CL_{INT}) were estimated by either pooling the data across different animals or recognising that the data comes from a population. Although the main aim of our study was the second one the first approach was undertaken to investigate the effect of assuming different priors on the posterior estimates as well as to provide a comparison with previous estimation using non-linear weighted least squares regression (14). In our study the number of animals was 24, the number of responses 12 (11 tissues and plasma) and we had between 3 and 6 measurements in plasma with one tissue concentration measured per rat per time point. Using the naïve pooled data approach the following model was assumed $y = f(x, \theta) + \varepsilon$, where the residual error is one and the same for all tissues. The prior Kp_b were either experimentally measured (15) or calculated by an *in silico* method proposed by Poulin and Thiel (18). Unfortunately, measures of Kp_b variances were not provided by either of these publications. These being the case, different

coefficients of variation were assumed for each Kp_b, ranging from 10 to 90%.

The following population diazepam WBPBPK models were fitted to the diazepam data.

$$y_{kij} = f_k\left(x_{ij}, \theta_i\right) + \varepsilon_{ij} \quad \varepsilon_{ij} \sim N\left(0, \sigma^2\right), \tag{1}$$

$$y_{kij} = f_k\left(x_{ij}, \theta_i\right) + \varepsilon_{kij} \quad \varepsilon_{kij} \sim N\left(0, \sigma_k^2\right). \tag{2}$$

Multiplicative error models were also investigated. Random effects were also added to estimate inter-animal variability, namely

$$\begin{aligned} y_{kij} = f_k\left(x_{ij}, \theta + b_i\right) + \varepsilon_{ij} \quad & \varepsilon_{ij} \sim N\left(0, \sigma^2\right) \\ \times b_i \sim N\left(0, D_i\right), \quad & D_i \sim (\mu, \Omega), \end{aligned} \tag{3}$$

$$\begin{aligned} y_{kij} = f_k\left(x_{ij}, \theta + b_i\right) + \varepsilon_{kij} \quad & \varepsilon_{kij} \sim N\left(0, \sigma_k^2\right) \\ \times b_i \sim N\left(0, D_i\right), \quad & D_i \sim (\mu, \Omega). \end{aligned} \tag{4}$$

Multiplicative error models, by applying a log transformation to the data and the predictions, were also investigated. In each of these models the priors of the fixed effects parameters had as mean the value given by the experimental values (15), with an assumed CV of 20%. Non-informative priors were assumed for both the residual variances (IG(0.01, 0.01)) and the random effects IW(0.01, 0.01). Consequently, to stabilise the estimation process informative priors were used for the random effects. Priors for $\Omega_{\mathrm{CL_{INT}}}$, which finally was our only random effect, were non-informative and specifically IW(0.5, 0.1).

Convergence following 4000 iterations with the first 2000 samples discarded was checked by visual inspection of the traces of the fixed effects and residual variance, as well as by the diagnostic checks in CODA.

Diazepam WBPBPK Model in Human

Eleven healthy volunteers, who have been described in a previous report (19) were included in the analysis. Briefly, single doses of 7 mg intravenous dose of diazepam were given to all subjects. Plasma samples were collected at 5, 15, 30, 45, 60 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 24, 30, 36, 48 and 72 hr.

The following model was used for fitting the human data

$$y_{kij} = f_k\left(x_{ij}, \theta + b_i\right) + \varepsilon_{ij} \quad \varepsilon_{ij} \sim N\left(0, \sigma^2\right) \quad b_i \sim N\left(0, D_i\right), \qquad (5)$$
$$D_i \sim (\mu, \Omega),$$

where CL_{INT} had an inter-individual variance component and Kp_b were fixed effects. Multiplicative error models, by applying a log transformation to the data and the predictions, were also investigated. As the MCMC simulations proved to be quite time consuming initially only 1000 iterations with the first 500 samples discarded with an informative CL_{INT} prior were performed. Subsequently convergence following 4000 iterations with the first 2000 samples discarded and 8000 iterations with the first 4000 samples discarded for the informative and non-informative CL_{INT} priors, respectively were checked by visual inspection of the parameters traces as well as by diagnostic checks in CODA.

RESULTS

Diazepam WBPBPK Model in Rat

The mean values of the two different sets of priors used in the naïve pooled data approach are plotted in Fig 3. CVs of 10, 20, 40, 70 and 90% were assumed to investigate their impact on the posterior Kp_b estimates. The mean values of Kp_b posterior estimates with the extreme CVs of 10 and 90% are plotted in Fig. 3b together with mean estimates coming from a previously published nonlinear weighted least squares regression (14). The corresponding numerical values are listed in Table II. Convergence was checked by examining trace plots (not shown) and it was accepted that stationarity of the chain was achieved after 4000 iterations. The posterior Kp_b estimates, after recognising that the data come from a population and assuming different residual variances in the different tissues (model from Eq. 2), are listed in Table III. Convergence was checked by the Raftery and Lewis criterion, trace plots inspection as well as Heidelberger and Welch stationarity and interval halfwidth tests (13). It appeared that stationarity of the chain was achieved. Attempts were made to add random effects to estimate the inter-individual variance in Kp_b using a multiplicative model as given in Eq. 4. Initially, vague priors were assigned to b_i but this resulted in difficulties in running the MCMC estimation. It was found that the posterior estimates of the random effects were heavily dependent on the priors, which resulted in abandoning this more complex model (Eq. 4) in favour of the fixed effects model with different residual variances for the tissues (Eq. 2).

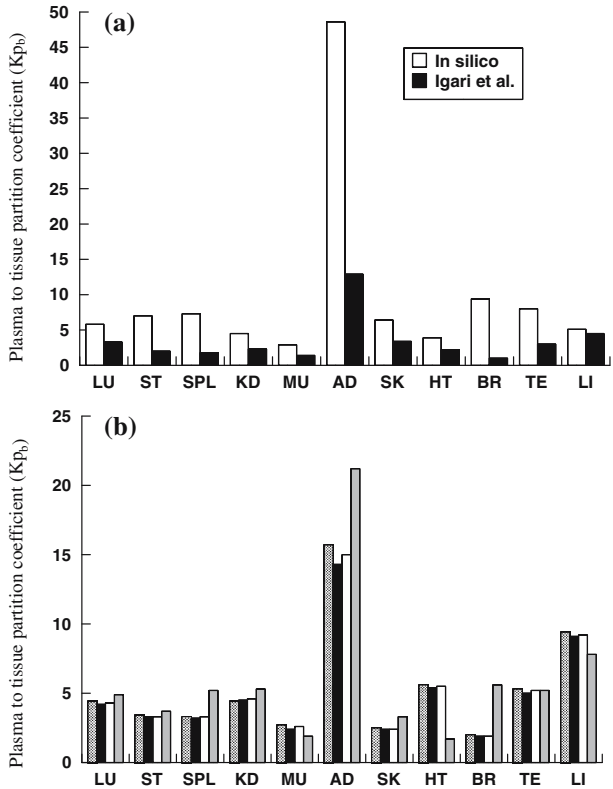

Fig. 3. (a) Rat prior mean Kp_b values from in silico calculations (white), reported by Poulin and Thiel (18) and experimental measurements (black) from (15), (b) Comparison of the rat posterior mean Kp_b estimates using priors derived from in silico calculations (shaded) reported by Poulin and Thiel (18), experimental values with either 10% CV (black) or 90% CV (white), measured in (15), as well as those estimated using nonlinear weighted least squares regression (dark grey), reported by Gueorguieva (14).

Using the full Bayesian approach, intrinsic clearance (CL_{INT}) was estimated simultaneously with the tissue-to-blood concentration ratios (Kp_b). The priors for the Kp_b were based again on those reported by (15) with an assumed 20% CV. Two scenarios were investigated, with non-informative and with informative priors for the CL_{INT}. The informative CL_{INT} prior was based on the estimate of total clearance from fitting the plasma data only in NONMEM, assigning all the clearance to hepatic metabolism and then calculating CL_{INT} assuming that the liver acts as a well-stirred compartment. Convergence was checked after 6000 iterations by examining the trace plots (Fig. 4), which show that stationarity

Table II. Naïve Pooled Data Approach: Rat Tissue-plasma Concentration Ratio Estimates

	Priors		Posteriors			Weighted nonlinear LS regression
Tissue Kps	Igari et al.	In silico[a]	Igari et al. 10% CV	Mean (sd) 90% CV	In silico mean (sd)	Mean (SE)
LU	3.3	5.8	4.2 (0.4)	4.3 (0.4)	4.4 (0.4)	4.9 (0.6)
ST	2[b]	7[c]	3.3 (0.2)	3.3 (0.2)	3.4 (0.2)	3.7 (0.4)
SPL	1.8	7.3	3.2 (0.2)	3.3 (0.2)	3.3 (0.2)	5.2 (0.6)
KD	2.3	4.5	4.5 (0.2)	4.6 (0.2)	4.4 (0.2)	5.3 (0.6)
MU	1.4	2.9	2.4 (0.6)	2.6 (0.6)	2.7 (0.6)	1.9 (0.2)
AD	12.9	48.6	14.3 (1.0)	15.0 (1.0)	15.7 (0.9)	21.2 (3.2)
SK	3.4	6.4	2.4 (0.8)	2.4 (0.7)	2.5 (0.7)	3.3 (0.4)
HT	2.2	3.9	5.4 (0.2)	5.5 (0.2)	5.6 (0.2)	1.7 (0.2)
BR	1.0	9.4	1.9 (0.4)	1.9 (0.4)	2.0 (0.4)	5.6 (0.6)
TE	3[b]	8[c]	5.0 (0.2)	5.2 (0.3)	5.3 (0.2)	5.2 (0.6)
LI	4.5	5.1	9.1 (0.3)	9.2 (0.4)	9.4 (0.4)	7.8 (0.9)
RE	4[d]	8[d]	3.2 (1.4)	3.2 (1.7)	4.1 (1.8)	18.2 (4.0)
σ^2	–[e]	–[e]	365.2 (33)	365.2 (33)	365.2 (33)	–

[a] CV 20% assumed.
[b] not reported in (15).
[c] could not be estimated from (18) due to lack of tissue composition data.
[d] value assumed based on preliminary simulations.
[e] non-informative prior *gamma* (0.001, 0.001).

Table III. Rat Posterior Population Kp_{bs} Mean (sd) and Residual Variance Mean (sd) Estimates

Tissue	Kp_b mean (sd)	Residual variance mean (sd)
LU	4.6 (0.3)	0.3 (0.1)
ST	4.9 (0.5)	0.5 (0.1)
SPL	3.4 (0.5)	0.8 (0.1)
KD	4.8 (0.3)	0.3 (0.1)
MU	3.7 (0.3)	0.5 (0.1)
AD	21.7 (2.9)	0.5 (0.1)
SK	2.9 (0.3)	0.5 (0.1)
HT	5.6 (0.6)	0.6 (0.1)
BR	2.1 (0.2)	0.7 (0.1)
TE	4.9 (0.3)	0.3 (0.0)
LI	8.8 (1.0)	0.6 (0.1)
ART	–	0.7 (0.1)

of the chain was achieved. The resulting posterior estimates are listed in Table IV and representative population fits to diazepam data are given in Fig. 5.

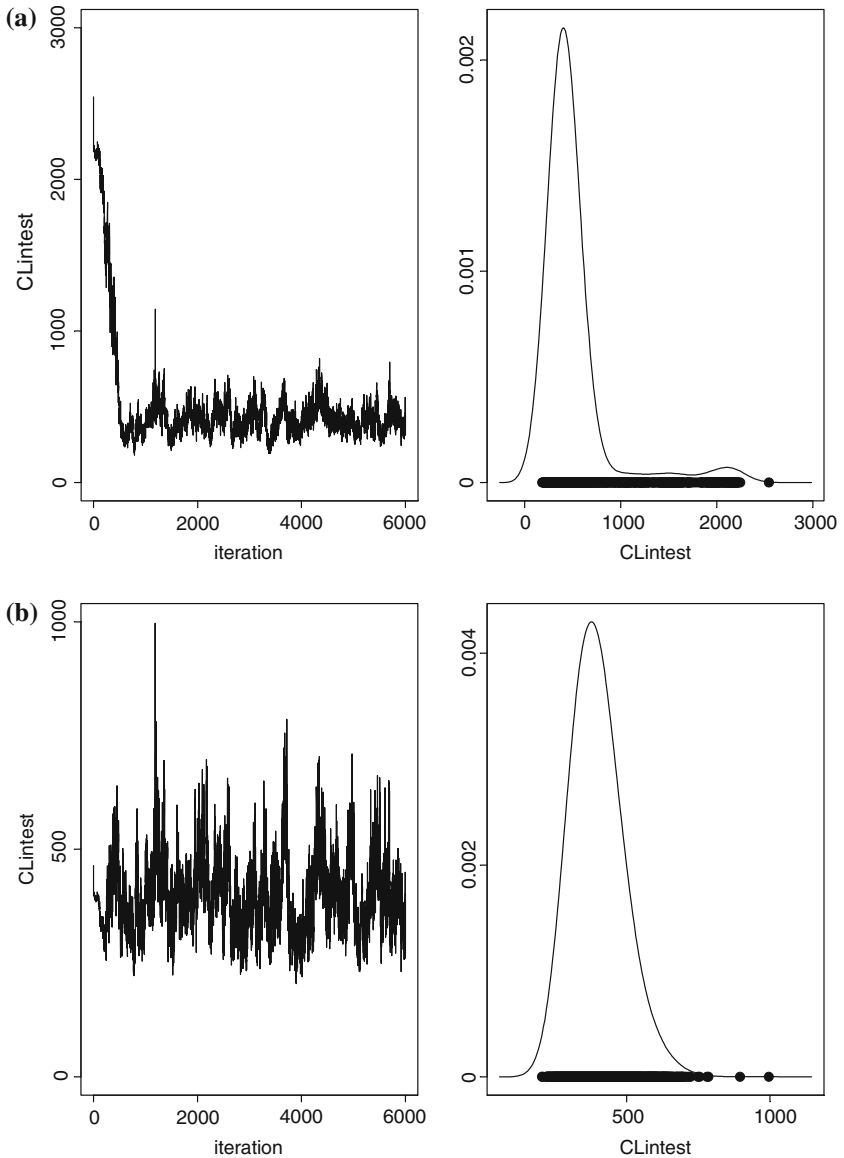

Fig. 4. Trace and density plots for rat CL_{INT} informative, (a) and non-informative (b) cases.

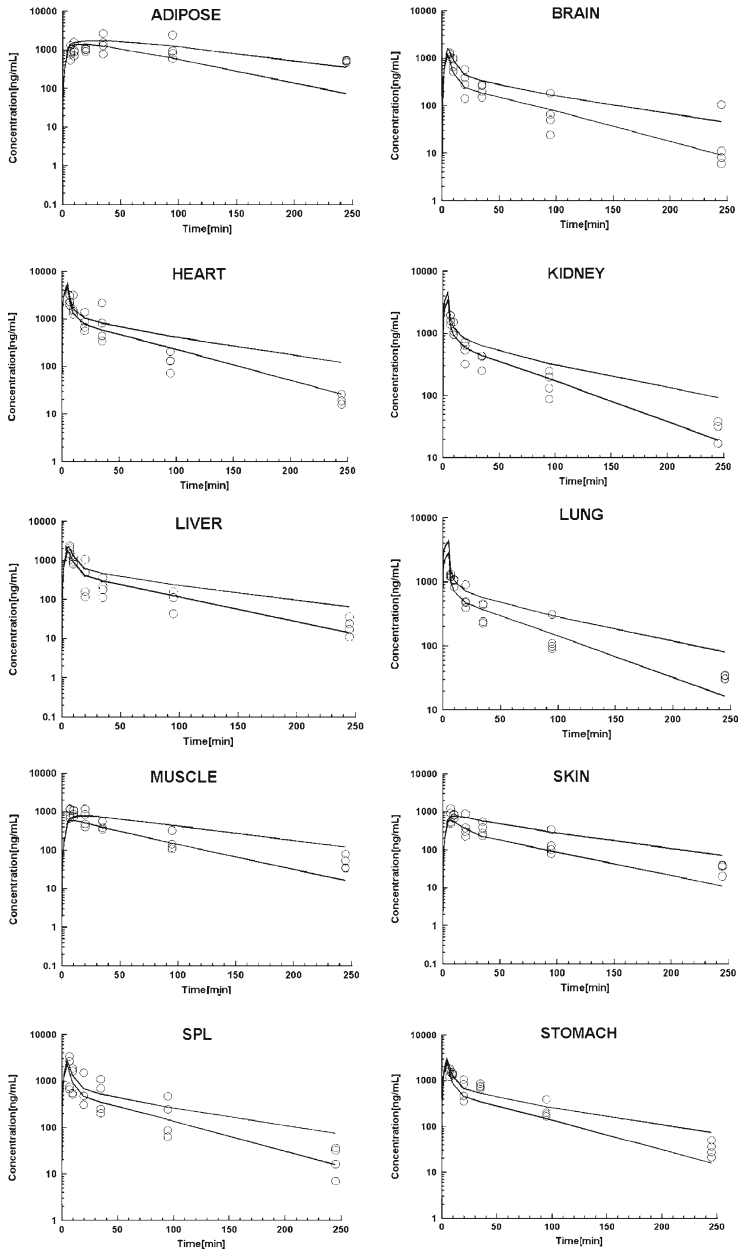

Fig. 5. 2.5 and 97.5% concentration-time profiles for diazepam disposition in tissues in rats (solid lines) and data (circles), using the full Bayesian approach.

Table IV. Rat Posterior Mean (sd) for Kp_{bs} and CL_{INT}, Inter-individual CL_{INT} Variance and Residual Variance Estimates with Informative and Non-informative Priors for CL_{INT}

	Non-informative case posteriors mean (SD)		Informative case posteriors mean (SD)	
	Tissue Kp_b	Residual variance σ^{2b}	Tissue Kp_b	Residual variance σ^{2b}
LU	4.5 (0.4)	0.3 (0.0)	4.6 (0.4)	0.3 (0.0)
ST	4.8 (0.5)	0.5 (0.1)	4.9 (0.5)	0.5 (0.1)
SPL	3.4 (0.5)	0.7 (0.1)	3.4 (0.5)	0.7 (0.1)
KD	4.7 (0.4)	0.3 (0.0)	4.7 (0.4)	0.3 (0.0)
MU	3.7 (0.3)	0.5 (0.1)	3.7 (0.3)	0.5 (0.1)
AD	21.0 (2.9)	0.5 (0.1)	21.0 (2.9)	0.5 (0.1)
SK	2.9 (0.3)	0.4 (0.1)	2.9 (0.3)	0.4 (0.1)
HT	5.4 (0.6)	0.6 (0.1)	5.4 (0.6)	0.6 (0.1)
BR	2.0 (0.2)	0.6 (0.1)	2.0 (0.2)	0.6 (0.1)
TE	4.8 (0.3)	0.3 (0.0)	4.9 (0.3)	0.3 (0.0)
LI	8.6 (1.7)	0.5 (0.1)	8.9 (1.7)	0.4 (0.1)
PLASMA	–	0.7 (0.1)	–	0.7 (0.1)
CL_{INT}	397.5 (86.1)	–	412.2 (85.8)	–
$\Omega^{a}_{CL_{INT}}$	0.5 (0.1)	–	0.5 (0.1)	–

[a] non-informative priors *gamma* (1, 0.01).
[b] non-informative priors *gamma* (0.1, 0.1).

Diazepam Human Population WBPBPK Model

The full diazepam WBPBPK model structure for rats (Fig. 1) was retained when analysing the human data. Human plasma data in 11 healthy volunteers following iv bolus dosing were available from (19, individual data, personal communication). The fraction unbound fu in man was reported to be 0.015 and the blood-to-plasma ratio R 0.65 (19). Similarly to other researchers we assumed that human Kp_{UB} values are identical with rat Kp_{UB} (15). Rat Kp_{UB} values were calculated from the previously estimated rat Kp by $Kp_{UB} = Kp_b/fu$. These Kp_{UB} values were used as informative priors for making inferences in man. Bayesian inferences were carried out with both informative and non-informative priors for CL_{INT}. The mean values for the CL_{INT} prior were based on a NONMEM fit of the plasma data, whereas the variance for the informative case was that obtained from NONMEM and for the non-informative scenario a CV of 70% was assigned. The prior and resulting posterior mean and standard deviation estimates for tissue Kp_{bs} and CL_{INT}, together with CL_{INT} inter- and intra-individual variance with informative priors for CL_{INT} and Kp_{Bs} obtained after 1000 and 4000 iterations, are listed in Table V. Prior and resulting posterior estimates with a non-informative prior for CL_{INT} and informative priors for Kp_{Bs} are given in Table VI.

Table V. Human Prior and Posterior Mean and Standard Deviation Estimates for Kp_{bs} and CL_{INT} Together with the Inter- and intra- individual Variance for CL_{INT} with **Informative priors** following 1000, 4000 and 8000 Iterations

Tissue	Priors	1000 Iterations	4000 Iterations	8000 Iterations
Kp_b	Mean (SD)	Posterior Mean (SD)	Posterior Mean (SD)	Posterior Mean (SD)
LU	0.7 (0.1)	0.7 (0.1)	0.7 (0.1)	0.7 (0.2)
ST	0.7 (0.1)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)
SPL	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)
KD	0.7 (0.0)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)
MU	0.5 (0.1)	0.3 (0.1)	0.2 (0.0)	0.2 (0.0)
AD	3.3 (0.4)	6.6 (1.8)	5.3 (1.8)	3.4 (0.3)
SK	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
HT	0.8 (0.1)	0.8 (0.3)	0.8 (0.2)	0.8 (0.2)
BR	0.3 (0.0)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)
TE	0.8 (0.1)	0.8 (0.1)	0.8 (0.2)	0.8 (0.2)
LI	1.4 (0.2)	0.8 (0.3)	0.8 (0.3)	0.7 (0.2)
CL_{INT}	70.8 (20)	23.1 (18.7)	41.9 (24.7)	74.8 (5.3)
$\Omega_{CL_{INT}}$	– [a]	0.15 (0.0)	0.15 (0.0)	0.16 (0.4)
σ^2	– [b]	0.7 (0.1)	0.6 (0.1)	0.5 (0.03)

[a] non-informative priors *gamma* (5, 0.01).
[b] non-informative priors *gamma* (0.01, 0.01).

Convergence was checked in all scenarios by examining the trace plots and the different diagnostic criteria in CODA. Trace plots after 1000 and 4000 iterations with informative priors for CL_{INT} and representative Kp_B are shown in Fig. 6a, b, respectively. Convergence was achieved for Kp_s, which was confirmed by Raftery and Lewis criterion and Heidelberger and Welch stationarity test. However this was not the case for CL_{INT} where after 4000 iterations a 'snake-like' appearance of its traces, indicative of nonconvergence is apparent. The trace plots for CL_{INT} after 8000 iterations with informative and non-informative priors are shown in Fig. 7a. Additionally representative trace plots after 8000 iterations for Kp_s are shown in Fig. 7b. It seems that convergence was achieved for all estimates in the two scenarios, which was also confirmed by Raftery and Lewis criterion and Heidelberger and Welch stationarity test. Human population plasma and brain predictions with informative and non-informative priors (superimposed) after 8000 iterations are shown in Fig. 8.

DISCUSSION

Our study illustrates the use of Bayesian statistics to estimate animal and population PK parameters though physiological modeling. The

Table VI. Human Prior and Posterior Mean and Standard Deviation Estimates for Kp_{bs} and CL_{INT} Together with Inter and intra individual Variance for CL_{INT} with **Non-informative priors** after 1000 and 8000 Iterations

		1000 Iterations	8000 Iterations
Tissue Kp_b	Priors Mean (SD)	Posterior Mean (SD)	Posterior Mean (SD)
LU	0.7 (0.1)	0.7 (0.2)	0.7 (0.2)
ST	0.7 (0.1)	0.7 (0.2)	0.7 (0.2)
SPL	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)
KD	0.7 (0.0)	0.7 (0.2)	0.7 (0.2)
MU	0.5 (0.1)	0.2 (0.0)	0.2 (0.0)
AD	3.3 (0.4)	3.4 (0.3)	3.4 (0.3)
SK	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)
HT	0.8 (0.1)	0.8 (0.2)	0.8 (0.2)
BR	0.3 (0.0)	0.3 (0.1)	0.3 (0.1)
TE	0.8 (0.1)	0.8 (0.2)	0.8 (0.2)
LI	1.4 (0.2)	0.8 (0.3)	0.7 (0.2)
CL_{INT}	70.8 (63.7)	75.7 (5.9)	75.0 (5.5)
$\Omega_{CL_{INT}}$	_[a]	0.16 (0.0)	0.16 (0.0)
σ^2	_[b]	0.5 (0.0)	0.5 (0.03)

[a] non-informative priors *gamma* (5, 0.01).
[b] non-informative priors *gamma* (0.01, 0.01).

method is very general and, we propose that it could be applied for integrating preclinical, human healthy volunteer and patient data into a WBPBPK model, which would allow continuous flow of information through the different stages of drug discovery and development. In this section, we discuss the methodological aspects of the study and address the results obtained and their importance.

Diazepam WBPBPK Model in Rat

A previously developed model for diazepam disposition (14) was used in a population Bayesian framework. The population WBPBPK model was fitted to the data and provided a good overall description of the concentrations in plasma and tissues. Although, we were not able to estimate inter- and intra-individual variability of the tissue-to-blood ratios Kp_{bs}, possibly due to either the data quality or the destructive nature of the experiments, we obtain estimates of inter- and intra-individual variability of intrinsic clearance as well as of the residual variance of each tissue. In our study the physiological parameters, blood flows and volumes, were held fixed to their mean values. Alternatively, we could have assigned prior distribution to them, as well as to the fraction unbound in plasma and blood-to-plasma ratios but, we believe that with the available data their

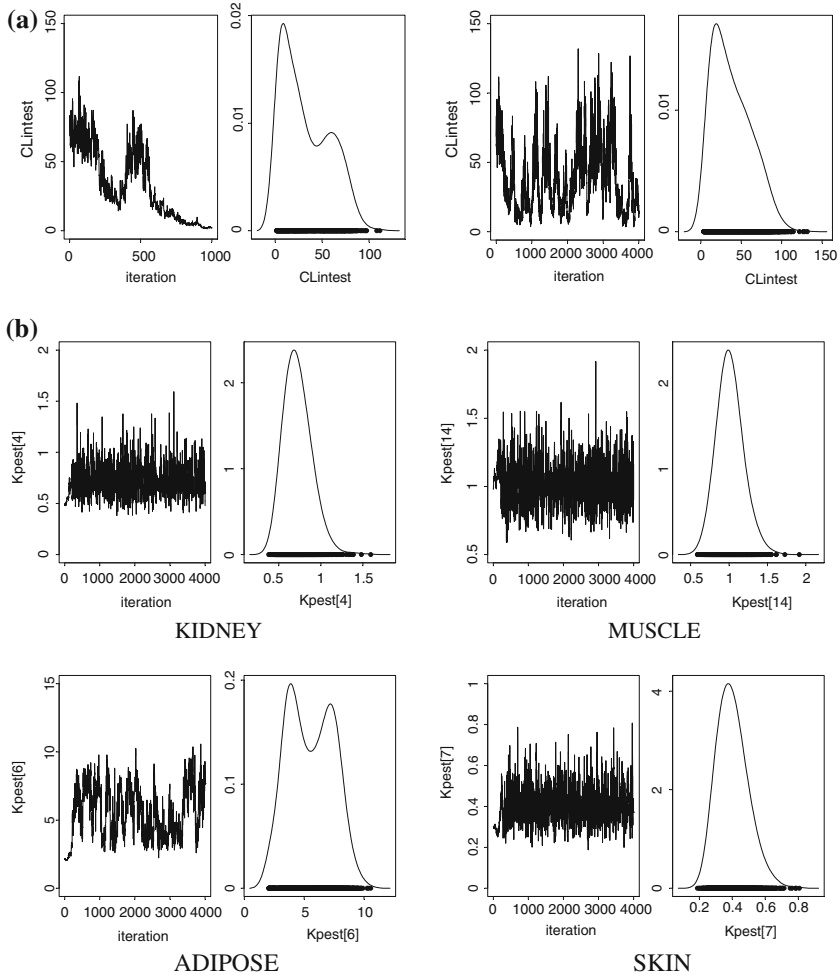

Fig. 6. Trace plots of, (a) human CL_{INT} with informative prior after 1000 and 4000 iterations and (b) Kp_s (KD, MU, AD, SK) after 4000 iterations in human.

estimation would not have been possible. The resulting mean and standard deviations estimates from the naïve pooled data approach with the two different sets of priors were almost identical, which indicated that the posterior in these cases was led by the likelihood rather than prior knowledge. That is also the reason why the mean estimates from the Bayesian naïve pooled data analysis were similar to those obtained from a previously reported nonlinear weighted least squares regression (14). The most

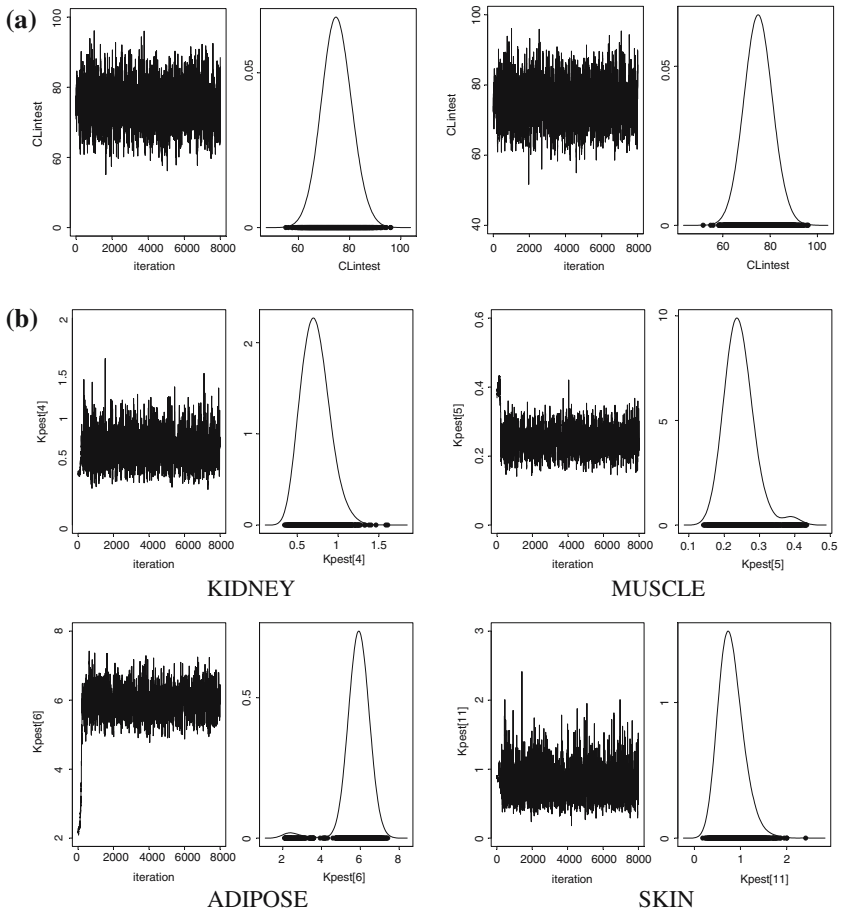

Fig. 7. Trace plots of (a) CL_{INT} with informative and non-informative prior after 8000 iterations and (b) representative Kp_s (KD, MU, AD and LI) after 8000 iterations in human.

pronounced differences in the mean estimates were observed for adipose and rest of the body Kp_s. Possibly any difference between the estimation techniques are reflected in these two estimates because adipose is the most slowly equilibrating tissue and RE accounts for the mass balance considerations, hence these two organs are most sensitive to any discrepancies between the estimation methods. However, as expected, the standard deviations for the Kp_s from the Bayesian analysis were smaller than the corresponding standard errors from the nonlinear regression analysis. Using population modelling with a different residual variance for each

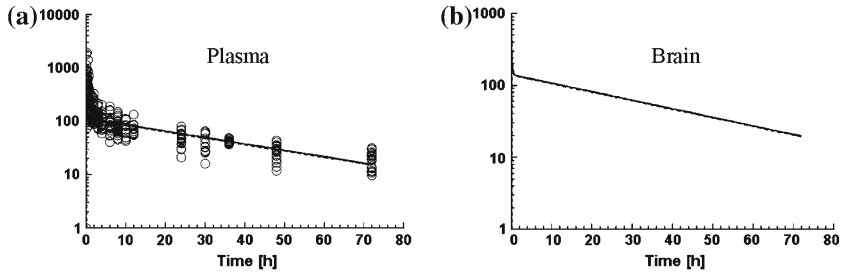

Fig. 8. Median human population plasma, (a) and brain, (b) predictions with informative (8000 iterations; dotted line) and non-informative (8000 iterations; solid line) priors. The two predictions with the informative and non-informative priors are superimposed.

tissue (Eq. 2) improved the estimate of the residual variability compared to when it was assumed to be one and the same for all tissues (Eq. 1). Initially, we assumed non-informative priors for Ω_{Kp_s} (Eq. 4) which led to the inability to run the estimation. We then made the priors more informative, which resulted in posterior estimates that were very similar to the priors, indicating that there was minimal information about the covariance in the data. As, we did not have any confidence in these prior values we chose the model structure given Eq. 2, with multiplicative error as our final rat WBPBPK model. It is possible that the mixed effects WBPBPK model was not identifiable, which could explain our inability to estimate inter-subject variability. However, we have not performed formal identifiability analysis and this issue requires future investigation.

Diazepam Human Population WBPBPK Model

Fitting the full WBPBPK to the human plasma data using Bayesian analysis proved to be a time consuming process (app 20 days for 8000 iterations). This was mainly due to the fact that as only plasma data was available, all the tissue data was treated as censored data, which led to a great number of iterations to calculate posterior estimates. The posterior estimates of the tissue-to-blood partition coefficients after 8000 iterations in all cases were almost identical to the priors. This was expected as there was no human tissue data to facilitate the estimation process. After 4000 iterations there were however two exceptions, namely adipose and liver Kp_{Bs}. The difference in liver Kp_b estimate can be explained by the high correlation to the intrinsic clearance, which was estimated simultaneously. The other exception was adipose Kp_b, which had a higher posterior estimate compared to its prior (see Table V, columns 3 and 4). A close inspection of the trace plots (Fig. 6b) showed that the adipose Kp_b had not converged. It was anticipated that the posterior estimate of $\mathrm{CL_{INT}}$

in the two scenarios would be in close agreement with that obtained from a NONMEM fit of the plasma data only. Although the estimates might not closely correspond due to the different structural models used it was expected that there would be agreement between the two, especially in the case with informative prior for the CL_{INT}. It was obvious that convergence was not achieved with 1000 iterations (Fig. 6a, left panel), which led to increasing the number of iterations to 4000. Although improvement was made convergence did not appear to be achieved (Fig. 6a, right panel). After reaching convergence, following 8000 iterations the posterior estimates of CL_{INT} in the two scenarios were very close to those estimated by NONMEM using only the plasma data. The population prediction where 8000 iterations were used improved the CL_{INT} convergence, and consequently produced population fitting close to the human data (Fig, 8a).

The posterior mean and inter-individual CL_{INT} were similar when informative and non-informative priors were used, however the estimated inter-subject variance for CLint is small. Possibly, the estimated inter-individual CL_{INT} variance will not be the same if, we had analysed only the plasma data and estimated the between-subject variance in total CL. Comparison between the two situations is not very easy, however, as there are a number of other factors, such variance in fraction of drug unbound in plasma, blood-to-plasma ratio, as well as in the WBPBPK model the estimation of Clint is but one parameter in this global model to accommodate the variability. In addition to these reasons we assume there is some form of model misspecification—presumably the model for the random effects. Clearly it is a poor estimate of the true inter-subject variability and that requires further investigation.

The WBPBPK model developed, tested and calibrated for rats was retained in human with estimating some of the parameters. Retaining the full model provides a complete representation of drug PK behaviour in human. Using a WBPBPK model in a Bayesian framework provides a flexible approach throughout drug discovery and development as, we can continuously update the model in the light of new information, whether physiological, disease or drug related.

APPENDIX: WBPBPK MODEL EQUATIONS

Non-eliminating tissues

$$V_T \frac{dC_T}{dt} = Q_T C_{\mathrm{ART}} - Q_T \frac{C_T}{K p_T}$$

$T = \mathrm{MU}, \mathrm{AD}, \mathrm{TE}, \mathrm{SK}, \mathrm{HT}, \mathrm{BR}, \mathrm{KI}, \mathrm{RE}, \mathrm{ST}, \mathrm{SPL}$

Liver

$$V_{\mathrm{LI}}\frac{dC_{\mathrm{LI}}}{dt} = Q_{\mathrm{HA}}C_{\mathrm{ART}} + \sum_j \frac{Q_j C_j}{Kp_j} - CL_{\mathrm{INT}}\frac{C_{\mathrm{LI}}}{Kpu_{\mathrm{LI}}} - Q_H\frac{C_{\mathrm{LI}}}{Kp_{\mathrm{LI}}}$$
$$= Q_{\mathrm{HA}}C_{\mathrm{ART}} - C_{\mathrm{LI}}\left(\frac{Q_H + CL_{\mathrm{INT}}f_{\mathrm{UB}}}{Kp_{\mathrm{LI}}}\right) + \sum_j \frac{Q_j C_j}{Kp_j}$$
$$j = \mathrm{ST},\ \mathrm{SPL}; \quad Q_H = Q_{\mathrm{HA}} + Q_{\mathrm{HP}} = Q_{\mathrm{HA}} + Q_{\mathrm{ST}} + Q_{\mathrm{SPL}},$$

where Q_{HA} hepatic arterial blood flow; Q_{HP} blood flow perfusing the hepatic portal vein which drains the intestines (SPL) and stomach (ST); $f_{\mathrm{UB}} = f_U/R$: fraction unbound in whole blood; $Kp_{\mathrm{U_{LI}}} = Kp/f_{\mathrm{UB}}$: tissue-to-unbound plasma partition coefficient.
Lungs

$$V_{\mathrm{LU}}\frac{dC_{\mathrm{LU}}}{dt} = Q_{\mathrm{VEN}}C_{\mathrm{VEN}} - Q_{\mathrm{LU}}\frac{C_{\mathrm{LU}}}{Kp_{\mathrm{LU}}},$$
$$Q_{\mathrm{LU}} = Q_{\mathrm{VEN}} = Q_{\mathrm{ART}}.$$

Arterial Blood

$$V_{\mathrm{ART}}\frac{dC_{\mathrm{ART}}}{dt} = Q_{\mathrm{LU}}\frac{C_{\mathrm{LU}}}{Kp_{\mathrm{LU}}} - Q_{\mathrm{ART}}C_{\mathrm{ART}}.$$

Mixed Venous Blood

$$V_{\mathrm{VEN}}\frac{dC_{\mathrm{VEN}}}{dt} = \sum_i \frac{Q_i C_i}{Kp_i} + Q_{\mathrm{H}}\frac{C_{\mathrm{LI}}}{Kp_{\mathrm{LI}}} - Q_{\mathrm{LU}}C_{\mathrm{VEN}} + R_{\mathrm{O}},$$
$$i = \mathrm{KI},\ \mathrm{MU},\ \mathrm{AD},\ \mathrm{SK},\ \mathrm{HT},\ \mathrm{BR},\ \mathrm{TE},\ \mathrm{RE}$$
$$R_{\mathrm{O}} = \begin{cases} \dfrac{\mathrm{Dose}}{T_{\mathrm{STOPPING\_INFUSION}}} & \mathrm{if} \quad t < T_{\mathrm{STOPPING\_INFUSION}} = 5\,\mathrm{min}, \\ 0 & \mathrm{if} \quad t \geqslant 5\,\mathrm{min}. \end{cases}$$

ACKNOWLEDGMENTS

Financial support for this project was provided by the following Centre for Applied Pharmacokinetic Research Consortium members: GlaxoSmithKline, Novartis, Pfizer, Roche, Servier and Eli Lilly. Dr David Lunn is acknowledged for supplying the beta-version of WinBUGS with ordinary differential equations solver and Dr David Greenblatt for providing diazepam human data.

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