Physiologically-Based Pharmacokinetics of Matrine in the Rat after Oral Administration of Pure Chemical and ACAPHA

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Abbreviations used are: PBPK, physiologically based pharmacokinetics; Q, blood flow; V, tissue volume; AUC, area under the concentration-time curve; PK, pharmacokinetics; BLPLR, blood/plasma ratio; CL, total body clearance; F, bioavailability factor; tlag, lag time; \( k_f \), fecal excretion rate constant; MPE, mean prediction error; \( V_{dss} \), volume of distribution at steady-state; LLOQ, lower limit of quantification; GC, gas chromatograph; GC/MS, gas chromatograph/mass spectrometry; HPLC/UV, high performance liquid chromatograph/ultraviolet; HPLC/MS, high performance liquid chromatograph/mass spectrometry.
ABSTRACT

ACAPHA®, a botanical drug for the treatment of human esophageal cancer in China, is under investigation as a lung cancer chemoprevention agent at the B.C. Cancer Agency. Little or no information is available on the pharmacokinetics of ACAPHA® in animals. The objectives of this study were: to examine the disposition kinetics of matrine, a bioactive marker of ACAPHA® in the rat, to develop a physiologically based pharmacokinetic (PBPK) model for pure matrine, and to characterize the absorption and clearance of crude matrine in ACAPHA®-treated rats using the PBPK model. Pure matrine (15 mg/kg) or crude matrine in the form of ACAPHA® (0.38 g/kg or 3.8 g/kg) was administered to the rat by gavages. The rats were sacrificed at different time points post-dosing. Blood and major organs were removed from the rat, extracted with toluene/butanol and quantified for matrine using gas chromatography-mass spectrometry. An eleven-compartment, flow-limited PBPK model of matrine was developed. The PBPK model was able to simulate closely the empirical data of rats treated with pure matrine. Since the absorption and clearance of crude matrine in ACAPHA®-treated rats could not be parameterized a priori; they were estimated by fitting the experimental data to the PBPK model. Results of the study show that pure matrine is absorbed and eliminated by the rat at faster rates than crude matrine. Moreover, ACAPHA® matrix may change the pharmacokinetics of matrine in the rat significantly. The PBPK model is a valuable tool to gain insights into the disposition kinetics of a botanical drug.
INTRODUCTION

ACAPHA® (a.k.a. Anti-tumor A) is a botanical drug prepared from six different Chinese herbs including *Sophora tonkinensis*, *Polygonum bistorta*, *Prunella vulgaris*, *Sonchus brachyotus*, *Dictamnus dasycarpus* and *Dioscorea bulbifera*. Previous clinical trial studies have shown that ACAPHA® treatment can reduce cancer progression in patients with marked esophageal dysplasia by 50% (Lin et al., 1990). ACAPHA® also decreases chemical-induced lung tumour multiplicity and tumor load by 40% and 70%, respectively in p53 transgenic mice that lack the Ink4a/Arf tumor suppressor genes (Zhang et al., 2004). ACAPHA® is currently under investigation as a potential chemoprevention agent for lung cancer in former smokers at the B.C Cancer Agency. Chemoprevention refers to the use of food, beverages or pharmacological agents in inhibiting, delaying or reversing the progression of carcinogenesis (Hong and Sporn, 1997).

In the present study, the pharmacokinetics (PK) of ACAPHA® is studied in the rat using matrine, a quinolizidine alkaloid of the *Sophora tonkinensis* roots as a bioactive marker (Srinivasan, 2006). Fig. 1 shows the chemical structure of matrine. The rationale of using matrine as a bioactive marker of ACAPHA® is as follows: (a) matrine and related chemicals have been shown to possess anti-neoplastic and anti-inflammatory activities towards leukemia cells (Zhu, 2001). These chemicals also are capable of stimulating immunological activity and inhibiting tumour growth in humans (Xu and Jiang, 1998; Chang, 1992), (b) although matrine constitutes <2% (w/w) of the *Sophora tonkinensis* roots, it is the only alkaloid found in the plasma/tissues of rats after ACAPHA® administration (Sit et al., 2004; Gao, 2007), (c) specific and sensitive analytical methods such as GC/MS (Sit et al., 2004), HPLC/UV (Wu et al., 2003), and...
HPLC/MS (Wang et al., 2005) have been developed to detect and quantify matrine in biological samples, and (d) matrine standard of high chemical purity is readily available from commercial sources.

Little or no information was available on the PK of crude matrine in animals or humans after administering the extract of an herbal product except the *Sophora Flavescens* Ait roots (Zhang et al., 2008; Wu et al., 2005). In contrast, the PK of pure matrine was studied extensively in animals (Wu et al., 2003; Luo and Xia, 1991; Wang and Huang, 1992) and humans (Wang et al., 1994). Plasma matrine PK in the rat was linearly related to the i.v. dose from 4-40 mg/kg (Wu et al., 2003). Matrine was found to distribute widely to the plasma/tissues of the rat after administering a single oral dose of the chemical (Luo and Xia, 1991). The time course of matrine concentrations in human (Wang et al., 1994) and rabbit (Wang and Huang, 1992) plasma could be described by a two-compartment pharmacokinetic model after i.v. administration.

We have developed a physiologically-based pharmacokinetic (PBPK) model (Gerlowski and Jain, 1983) to study the pharmacokinetic properties and tissue distribution of matrine in the rat after administration of pure matrine. The PBPK model is based largely on the actual anatomy and physiology of the rat and the physicochemical properties of matrine. Although the PBPK model has been used widely to study the PK of individual environmental chemicals (Clewell and Andersen, 1985) and/or their mixtures (Haddad et al. 1999), relatively few PBPK models have been developed for the prescription drug (Thiel et al., 2003). Even fewer PBPK models are developed for the botanical drug. As far as we know, only the following botanical
drug PBPK models have been reported to date: soy isoflavones (Law, 2007a; Schlosser et al., 2006), tea catechins (Law, 2007b; Law, 2006), caffeine (Ginsberg et al. 2004), sophoridine (Hu and Huang, 1995), and glycyrrhizin acid (Ploeger et al., 2000). PBPK models had been used to extrapolate dose-response relationships between species, routes of administration, and dosage regimens (Nestorov, 2003). They also were used to predict the concentration-time profiles of several bioactive chemicals simultaneously in humans after administration of an herbal product (Law, 2007a, b).

The objectives of the present study were: to study matrine tissue distribution in the rat after administering pure matrine or crude matrine in the form of ACAPHA®, to develop a PBPK model of matrine based on the kinetic data derived from pure matrine-treated rats, and to characterize crude matrine absorption and clearance in ACAPHA®-treated rats using the PBPK model.
MATERIALS AND METHODS

Chemicals

Matrine was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Chemical purity of matrine, determined by gas chromatography-mass spectrometry (GC/MS) and high performance liquid chromatography, was >99%. Pure matrine was fine white powders which dissolved readily in water and common organic solvents. Matrine-14, 14-d$_2$ was synthesized in our laboratory using a chemical reaction which could replace >97% of the hydrogen at position 14 of the matrine molecule with deuterium (Sit et al. 2004). ACAPHA$^*$ was a gift from the Global Cancer Strategies Ltd. (British Columbia, Canada); it was a mixture of dark brown powders and amorphous solids. ACAPHA$^*$ was standardized to 0.4 % of pure matrine (w/w) by the manufacturer. No oxymatrine was present in the final product.

Animals

Male Sprague-Dawley rats (325-375 g) were purchased from Charles River Laboratories, Inc. (Quebec, Canada). The rats were maintained on a constant light-dark cycle with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Tap water and food were provided ad libitum. The rats were used in the tissue distribution studies after a 7-day acclimation period. The procedure associated with animal care and experimentation was carried out according to the Canadian Council on Animal Care Guidelines and with formal approval of the Simon Fraser University Animal Care Committee.
Preparation of ACAPHA® suspension and matrine solution

Pure matrine was dissolved in distilled water as a 1 mg/ml solution before being administered to the rat. ACAPHA® did not dissolve in water completely; it was prepared as aqueous slurry (25 mg/ml or 250 mg/ml) which was dispersed by ultra-sonication before use.

Animal studies

Rats were fasted overnight but allowed free access to water before pure matrine or ACAPHA® administration. Groups of rats (N=18) were given a single dose of ACAPHA® (3.8 g/kg or 0.38 g/kg) or pure matrine (15 mg/kg) by gavages. Since ACAPHA® was standardized to 0.4 % matrine (w/w), a 3.8 g/kg ACAPHA® dose was equivalent to a dose of 15 mg/kg pure matrine. After dosing, three rats from each group were randomly selected and sacrificed by CO₂ asphyxiation at different time points: 0, 0.25, 1, 2, 4, and 6 hr for the 0.38 g/kg ACAPHA® study; 0, 0.25, 1, 3, 6, and 12 hr for the 3.8 g/kg ACAPHA® and the 15 mg/kg pure matrine studies. A blood sample was withdrawn immediately from the rat by cardiac puncture and centrifuged at 3000 x g for 5 min to collect the plasma. Major organs such as the heart, liver, spleen, lung, kidney, fat, muscle, and brain also were collected from the rat. The organs were rinsed briefly in distilled water, wiped dry with paper, and stored in vials at -20 °C until analysis. Matrine was stable chemically under these storage conditions for at least one month.

Blood to plasma ratio

About 5 ml of freshly drawn rat blood was mixed with a standard matrine solution to a final concentration of 10 or 100 µg/ml. After incubation at 37 °C for 30 min, aliquots of the blood samples were removed and centrifuged at 3000 x g for 5 min at room temperature to
obtain the plasma. Preliminary studies indicated that matrine equilibrium was established between the blood and the plasma in 20 min under these experimental conditions. Matrine concentrations in the plasma samples were measured by GC/MS (Sit et al., 2004). The concentrations in blood were assumed to be the theoretical concentration. Blood:plasma concentration ratio was calculated by dividing matrine concentration in the whole blood with those in the plasma.

**Extraction of matrine from plasma and tissue samples**

Plasma/tissue samples were extracted and quantified for matrine according to the procedure of Sit et al., (2004). Briefly, about 1.0 g of tissue sample was weighed and homogenized in 3 ml of distilled water with a Kinemetica GmbH homogenizer (PCU-2-110, Switzerland). A 1-ml aliquot of the tissue homogenate or plasma was removed and mixed with 250 ng of matrine-14,14-d$_2$ (the internal standard), 3 ml of toluene:butanol (v/v 7:3), and 0.5 ml NaOH (1 M) in a centrifuge tube. The mixture was shaken on a mechanical shaker for 20 min and centrifuged at 3000 rpm to separate the layers. The organic layer was removed and extracted with 0.5 ml HCl (0.25 M). The organic layer was discarded and the remaining aqueous layer was neutralized with 0.5 ml of 1.0 M NaOH before being extracted by 200 μl of toluene:butanol (v/v 9:1). The organic layer was separated by centrifugation and analyzed by GC/MS (Sit et al., 2004). Matrine concentration in the tissue sample was calculated after correcting for the dilution volume and tissue weight.

**Gas chromatograph/mass spectrometric determination of matrine**

A Hewlett-Packard 5890 series II gas chromatograph coupled to a 5971 mass spectrometric detector was used to quantify matrine in the toluene:butanol (v/v 9:1) extract.
Chromatographic separation was performed with a 5% diphenyl-95% dimethylpolysiloxane capillary column (30 m x 0.25 mm x 0.25 μm, HP-5 MS). Helium was used as the carrier gas. The initial oven temperature was set at 110 °C, maintained for 1 min and then increased to 220 °C at a rate of 30 °C/min and maintained for 1 min. The temperature was further increased to 300 °C at a rate of 15 °C/min and maintained for 3 min. Ionization was performed under electron impact ionization with 70 eV. Matrine and matrine-14,14-d$_2$ were quantified using the $m/z$ 248 and $m/z$ 250, respectively (Sit et al., 2004). A linear response was obtained with matrine standards over the range of 10-500 ng/ml. The lower limit of quantitation (LLOQ) of the GC/MS method for matrine was 12 ng/ml of plasma or tissue homogenate. A plasma/tissue sample was assigned to one half of the LLOQ when it was below 12 ng/ml in concentration. Thus, if two consecutive time points were below the LLOQ, the first and second time points would be given 6.0 ng/ml and 0.0 ng/ml concentrations, respectively.

**Model structure**

The PBPK model of matrine for the rat consisted of eleven flow-limited compartments including the lung, heart, kidney, fat, muscle, brain, liver, spleen, gut, blood, and the rest of body (Fig. 2). The rest of the body compartment includes all other tissues which had not been identified in the model i.e., the skin, bone, eye, prostate gland, etc. An eleven-compartment PBPK model was used in the present study because ACAPHA$^*$ was a multi-target therapeutic/prophylactic agent with different disease treatment endpoints. A linear, first-order input function was used to describe oral absorption of matrine (see Appendix). Matrine metabolism was not considered in the present PBPK model since no matrine was metabolized by the rat (Xie et al., 1983; Gao, 2007). Matrine was excreted mainly by the kidney of the PBPK
model (Fig. 2) since the rat excreted about 52% and 0.36% of the dose in the urine and faeces, respectively within 24 hr after matrine administration (Luo and Xia, 1991).

**Model parameters**

*Physiological parameters.* All physiological parameters are parameterized *a priori* (Table 1). The tissue volume and blood flow were expressed as a fraction of total body volume and cardiac output (CO), respectively and were taken from the literature (Luttringer at al., 2003). The total blood volume was divided into a two-thirds venous pool and one-third arterial pool. Gut contents was assumed to be 0.014 L for rats weighing 0.25 kg (Angelo and Pritchard, 1987).

*Tissue:plasma partition coefficients.* *In vivo* tissue/plasma partition coefficients (Table 1) were estimated using the area method of Gallo et al., (1987) as follows: the kinetic data of rats after administering a single oral dose of 15 mg/kg pure matrine were analyzed using the non-compartmental approach of WinNonlin*®* (Scientific Consulting, Inc. Version 1.5). The area under the concentration-time curve (AUC) was calculated using the logarithmic trapezoidal rule. The tissue/plasma partition coefficient of a specific tissue was calculated by dividing the AUC of the tissue with the AUC of plasma.

*Model parameterization for pure matrine.* The blood/plasma ratio (BLPLR) of matrine in the rat was determined experimentally to be $0.97 \pm 0.06$ (mean $\pm$ S.D.) at our laboratory. An absorption rate constant ($k_a$), $0.32$ hr$^{-1}$ was estimated by fitting the plasma concentration-time data of Wu at al., (2003) to a one-compartment pharmacokinetic model with WinNonlin*®* (Scientific Consulting, Inc. Version 1.5). Renal clearance (CL) was found to range from 1.4 to 1.6 L/hr-kg: A CL of 1.4 L/hr-kg was obtained by dividing the bolus *i.v.* dose with the plasma AUC reported by Wu et al., (2003). A second CL, 1.6 L/hr-kg was calculated from the renal excretion
rate of matrine in the study of Luo and Xia (1991). Hepatic extraction ratio (EH) was assumed to be 0.3% of the administered dose since only 0.27% of an orally administered dose was excreted in the bile in 12 hr (Luo and Xia, 1991). Moreover, matrine was not metabolized by the rats (Xie et al. 1983, Gao 2007). Faecal excretion rate constant (kf) was assumed to be 1/transit time of the small intestine; transit time of small intestine was obtained from Davies and Morris (1993). The experimental bioavailability factor (F) was assumed to be 0.44 of the administered dose since approximately 44% of an orally administered dose was absorbed into the systemic circulation (Wu et al., 2003). It should be pointed that F in the present study had no relationship to the absolute bioavailability of matrine (Anderton et al., 2004). Adjustments were made on the parameters with available experimental data during model development. However, once the parameter values were finalized (Table 2), no further adjustment was allowed.

**Model validation**

The PBPK model was validated by comparing model simulated results with the experimental data of Wistar rats after administering 40 mg/kg of pure matrine as a single oral dose or as an i.v. bolus injection (Wu et al., 2003). The PBPK model was assumed to be validated if model simulation described closely the experimental data. Since we were unsuccessful in obtaining the original data from these authors, the concentration-time data were read digitally with the ImageJ (Rasband, 2008). The parameter values of pure matrine (Table 2) were used to run the PBPK model in the validation study.

**Computer simulation and data fitting**
**Computer simulation.** The differential and algebraic equations describing the movement of matrine through the rat were formulated as a computer program. Mass balance differential equations for the model and the definition of the algebraic terms were given in the Appendix. The set of differential and algebraic equations incorporating the parameters in Tables 1 and 2 were solved numerically with the aid of AcslXtreme® 2.0.1.2 (AEgis Simulation, Inc.).

**Data fitting.** The $k_a$, F and CL values of crude matrine after ACAPHA® administration could not be parameterized a priori; they were estimated by fitting the experimental data to the PBPK model. The optimization procedure was carried out with all the parameter values of the pure matrine PBPK model fixed (Tables 1 and 2) except $k_a$, F and CL. Thus, $k_a$, F and CL were adjusted manually at first to get a feeling of the parameter effects on the simulation results. The adjusted values were then fitted to the experimental data using the maximized log likelihood function of AcslXtreme® OptStat (AEgis Simulation, Inc.). Upper and lower bound values were also assigned to each of the $k_a$, F and CL parameters to ensure the final parameter values were biologically and mathematically logical.

**Statistical and sensitivity analyses**

**Statistical analysis.** Mean deviations between tissue concentrations predicted by the PBPK model ($C_{pred}$) and the experimental data ($C_{exptl}$) were expressed as the mean prediction error, MPE according to the following equation (Wu, 1995):

$$\text{MPE} \% = \frac{\Sigma}{N}\left[100\frac{C_{exptl} - C_{pred}}{C_{exptl}}\right]$$

Prediction error was analogous to the calculation of “N-fold” deviations; $N$ was the number of
time points on the tissue compartment.

Sensitivity analysis. Sensitivity was calculated by the following equation (Clewell et al. 1994):

\[ \frac{\partial \ln R}{\partial \ln X} \]

where, R was a model output and X was the parameter for which the sensitivity was assessed.

The sensitivity coefficients were log-normalized to the parameters and the log-normalized sensitivity coefficients (LNSC) were calculated by AcslXtreme 2.0.1.2 (AEgis Simulation, Inc.) using the central difference method. The LNSC represented the percentage change in an output value associated with the percentage change in the input parameter. Since matrine concentration in the lung and the blood compartments were the outputs of concern in our study, the sensitivities of lung and blood matrine concentrations were examined with respect to the following parameters: F, \( k_a \), \( t_{lag} \), EH, CL, BLPLR, and \( k_f \). Thus if the absolute value of a LNSC were high, the parameter would have an important effect on the model prediction. If the absolute value of a LNSC were close to zero, the model parameter would have only a small effect on model prediction (Clewell et al., 1994).
RESULTS

Matrine tissue distribution in the rat was studied using a single oral dose of pure matrine (15 mg/kg) or ACAPHA® (0.38 g/kg or 3.8g/kg). Preliminary results showed that the 3.8 g/kg ACAPHA® study was limited by the volume of slurries that could be administered to the rat whereas the 0.38 g/kg ACAPHA® study was restricted by the low levels of matrine found in the plasma/tissues of the rat and the LLOQ of the GC/MS method. Since pure matrine was readily soluble in water, it was administered as an aqueous solution to the rat.

Fig. 3 depicts the time course of model-simulated matrine concentrations and the mean experimental data of the rat after pure matrine or ACAPHA® administration. The concentration-time data of individual plasma/tissue displayed a typical kinetic profile of an orally administered drug; matrine concentrations rose rapidly during the absorption phase and declined slowly in the post-absorptive phase. The 3.8 g/kg ACAPHA® study had about 10-fold higher plasma/tissue matrine concentrations than the 0.38 g/kg ACAPHA® study of which the matrine concentrations were the lowest in the present studies. Interestingly, matrine concentrations in the 3.8 g/kg ACAPHA® study were initially lower than the pure matrine study, but became higher at about 2 hr (kidney) or 4 hr (plasma and other tissues) post-dosing (Fig. 3). The kinetic profiles of pure matrine and 3.8 g/kg ACAPHA® studies were different although both studies were conducted with the same matrine dose equivalents. Thus, the kinetic profile of a specific tissue in the 0.38 g/kg ACAPHA® study but not the 3.8 g/kg ACAPHA® study was somewhat parallel to that of the corresponding tissue in the pure matrine study (Fig. 3). Nevertheless, the PBPK model was able to simulate all pure matrine and ACAPHA® datasets closely (Fig. 3).
Tables 1 and 2 list the final pharmacokinetic parameters that are used to implement the PBPK model. Two different sets of $k_a$, F and CL values (Table 2) were required to implement the PBPK model after pure matrine and ACAPHA® administration since they were essentially two different matrine formulations. Two different sets of F and CL values (Table 2) also were needed to simulate the kinetic profiles of the rat after administration of 3.8 g/kg and 0.38 g/kg ACAPHA® since they were the “best-fitted” parameter values of the experimental data. An attempt was made to simulate both the high dose and the low dose ACAPHA® data with a single set of F and CL values but with very little success since a good fit to the experimental data could be achieved only with the high dose or the low dose study but not both studies concurrently (data not shown).

The PBPK model of matrine in the rat was validated using the empirical data of Wu et al., (2003). As shown in Fig. 4, the PBPK model was able to predict closely the time course of matrine concentrations in the plasma of rats after administering 40 mg/kg of pure matrine via the i.v. (Fig. 4a) or the p.o. (Fig 4b) route.

The mean prediction error (MPE) was used to confirm our initial observation that model predictions described closely the experimental data (see Fig. 3). Deviations within a factor of two between model prediction and experimental data i.e., a MPE absolute value of <50%, were taken as the evidence that the PBPK model described the empirical data closely (Poulin and Thiel, 2002). As shown in Table 3, all MPE absolute values were <50% between the high dose and low dose ACAPHA® studies. Similarly, all MPE absolute values were <50% in the pure matrine study except those of the spleen and lung.
Table 4 summarizes the LNSC of the pure matrine study. The LNSC of the CL, $k_a$, BLPLR, and EH parameters had negative values whereas the LNSC of the F and $t_{lag}$ parameters had positive values. We assigned the tested parameters to high (>0.5), medium, and low (<0.05) impact groups according to the LNSC absolute values (Table 4). Since the LNSC of CL, BLPLR and F were >0.5 in absolute values, they had the most impact on the predicted matrine concentrations in the lung and/or blood. Similarly, $k_a$ was found to have a moderate impact on the predicted matrine concentrations in the blood and lung. Since the LNSC absolute values of the EH, $t_{lag}$ and $k_f$ parameters were <0.05, these parameters had only minor to negligible impacts on the predicted matrine concentrations in the blood and lung.
DISCUSSION

Our PBPK model provides a simple description on the movement of matrine in the rat after pure matrine or ACAPHA® administration (Fig. 2; Table 2). A comparison of the predicted versus actual concentration-time profiles (Fig. 3) show that the PBPK model is able to simulate the empirical data closely (Table 3). A surprise finding of our study is that different plasma/tissue kinetic profiles are found in the pure matrine and 3.8 g/kg ACAPHA® studies although the matrine dose equivalents are the same in both studies (Fig. 3). An explanation for the different kinetic profiles in these studies is not readily available but may be related to the diminished matrine absorption (k_a) and/or clearance (CL) in the 3.8 g/kg ACAPHA® study because of interactions between ACAPHA® matrix and the gastrointestinal tract or the kidney: (a) k_a. As shown in Table 2, the final pure matrine k_a value of the PBPK model is 6.5 hr⁻¹. It is about 20-fold higher than the 0.32 hr⁻¹ k_a value derived by fitting the plasma concentration data of Wu et al., (2003) to a one-compartment pharmacokinetic model. Different pure matrine k_a values are observed in these studies probably because they are conducted with different doses, rat species, model structures and/or PK approaches. Table 2 also shows that pure matrine has a higher k_a value than crude matrine in ACAPHA®. This probably is due to the different water solubility of pure matrine and crude matrine. Linnankoski et al., (2006) have shown that drug absorption is determined mainly by the dissolution rate in the gastrointestinal fluids. Since pure matrine dissolves in water readily, it is rapidly absorbed by the rat. However, because crude matrine is physically trapped or bound to ACAPHA® matrix, it does not dissolve in water easily. Thus crude matrine is absorbed at a slower rate than pure matrine by the rat. An alternate
explanation for a small crude matrine $k_a$ value may be that ACAPHA\textsuperscript{*} matrix is able to inhibit active matrine transport and/or compete with matrine for absorption in the gastrointestinal tract. (b) $CL$. The final pure matrine $CL$ value of the PBPK model is shown to be 1.2 L/hr-kg (Table 2). It is very close to the 1.4 L/hr-kg and 1.6 L/hr-kg $CL$ values derived from the experimental data of Wu et al., (2003) and Luo and Xia (1991), respectively. In contrast, the final crude matrine $CL$ values of the PBPK model are 0.35 L/hr-kg and 0.20 L/hr-kg, respectively in rats treated with 0.38 g/kg and 3.8 g/kg ACAPHA\textsuperscript{*} (Table 2). The crude matrine $CL$ values are very close to the 0.1 L/hr-kg $CL$ value reported for the *Sophora flavescens* Ait root extract (Zhang et al., 2008). Together these studies show that pure matrine $CL$ is consistently higher than crude matrine $CL$. Perhaps the matrices of ACAPHA\textsuperscript{*} and *Sophora flavescens* Ait root extract are able to inhibit active matrine secretion and/or induce matrine re-absorption by interacting with the canalicular transporters and/or P-glycoproteins of the kidney. Our assumption that matrine is being actively secreted into the kidney is supported by the 1.2 L/hr-kg $CL$ value of pure matrine (Table 2) which is about 4-fold higher than the 0.31 L/hr-kg glomerulus filtration rate of the rat (Davies and Morris, 1993).

Distribution is another process which has an impact on the PK of matrine in the rat. The kinetic parameter used to describe matrine distribution is the volume of distribution at steady-state ($V_{dss}$), the equivalent plasma volume in which matrine is distributed to the rat body. We have employed two different methods to calculate the $V_{dss}$ in the present study: (a) A $V_{dss}$ of 1.45 L is estimated for pure matrine using the non-compartmental approach to analyze the mean plasma concentration-time curve (Fig. 3a), and (b) A $V_{dss}$ of 1.1 L also is calculated by adding the products of each tissue volume and the corresponding partition coefficient and the
plasma volume of the PBPK model (Benowitz et al., 1975). The closeness of these $V_{ds}$ values suggests that the AUC-based partition coefficient estimation method (Gallo et al., 1987) provides reasonable estimates of the true value in the rats. It should be pointed out that both $V_{ds}$ values are much larger than the average body weight of the rat used in the present study, which would be indicative of extensive matrine binding with the plasma/tissue proteins. However, since the BLPLR ratio is very close to one (Table 2), protein binding does not appear to be a logical explanation for the large $V_{ds}$ in our study. A more likely explanation may be that the active transporters also are involved in the tissues/organ uptake of matrine. Thus the liver and kidney which accumulate high levels of matrine (Figs. 3b and 3h) also are known to have high active transport activities. Other possible but less likely explanation may be that matrine is bound preferentially to the different tissue macromolecules such as the DNA/RNA (Zhang et al. 2004).

The PBPK model requires 40 or more parameters for its implementation and not all parameters affect the simulation results equally. We have used the analytical sensitivity analysis (Clewell et al., 1994) to identify the parameters which have significant impacts on the model prediction. Our results show that $CL$, BLPLR, $k_a$ and $F$ are sensitive parameters in predicting matrine concentrations in the lung and/or blood at different time intervals post-dosing (Table 4). These results are in agreement with the finding that the LNSC of PBPK model parameters are complex functions of dosage, animal species, model structures and dose surrogates of the target tissues (Clewell et al., 1994). Table 4 also shows the LNSC of $CL$, $k_a$, BLPLR, and $EH$ are negative values whereas the LNSC of $F$ and $t_{lag}$ are positive values. These
indicate an increase in lung and/or blood matrine concentrations as $F$ and $t_{lag}$ increase or as $CL$, $k_a$, BLPLR, and EH decrease. Since $CL$ and $k_a$ are smaller values in the ACAPHA* studies than the pure matrine study (Table 2), ACAPHA* -treated rats would accumulate more matrine in the lung compare to pure matrine-treated rats. Indeed, mean matrine concentrations in the lung of the 3.8 g/kg ACAPHA* study are higher than those of the pure matrine study at about 4 hr post-dosing and thereafter (Fig. 3). Thus, ACAPHA* may be a better lung cancer chemoprevention agent than pure matrine. Further studies are required to determine if the amount of matrine accumulated by the lung after ACAPHA* administration can inhibit tumor growth while at the same time causing no intolerable toxicity to the normal tissues.

A PBPK model of sophoridine has been developed in the rat after a bolus i.v. injection of the chemical (Hu and Huang, 1995). Sophoridine is a stereoisomer of matrine. The BLPLR, $CL$ and $V_{dss}$ parameter values of the sophoridine PBPK model are 1.0, 1.35 L/hr-kg and 0.89 L, respectively (Hu and Huang, 1995) which are very close to the corresponding parameter values in our pure matrine study (Table 2). As a stereoisomer of matrine, sophoridine is expected to have similar physicochemical properties and model parameter values as matrine. But the $V_{dss}$ of sophoridine (0.86 L) is slightly less than the $V_{dss}$ in our pure matrine study (see above). However, in agreement with our study, the $V_{dss}$ of sophoridine is much larger than the average body weight (0.23 kg) of the rat used in the study (Hu and Huang, 1995).

In summary, our investigations have demonstrated the usefulness of the PBPK model in gaining insights into the physiological, biochemical and physical factors that play important roles in the observed kinetic behaviour of matrine in the rat after pure matrine or ACAPHA*
administration. The PBPK model framework developed in this study also can be used to study the PK of dietary supplements, nutraceuticals, and functional foods.
REFERENCES


FOOTNOTE

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LEGENDS TO FIGURES

Figure 1. Chemical structure of matrine.

Figure 2. Schematic diagram of the physiologically based pharmacokinetic model used to simulate the disposition of matrine in plasma and tissues of rats following administration of pure matrine or ACAPHA®. C represents matrine concentrations, ng/g or ml; Q represents plasma flow rates, L/hr. Subscripts refer to tissues (see Appendix).

Figure 3. Comparison of model-simulated and experimental matrine concentrations in the tissue/plasma of rats after receiving pure matrine or ACAPHA®. (a) plasma, (b) kidney, (c) spleen, (d) brain, (e) heart, (f) muscle, (g) lung, (h) liver, and (i) fat.

● represents experimental data of rats receiving 15 mg/kg of pure matrine; □ represents experimental data of rats receiving 3.8 g/kg of ACAPHA®; ■ represents experimental data of rats receiving 0.38 g/kg of ACAPHA®. Data points represent means ± SD of the experimental plasma/tissue concentrations from 3 different rats; ______ represents model-simulated matrine concentrations of rats receiving 15 mg/kg pure matrine; represents model-simulated matrine concentrations of rats receiving 3.8 g/kg ACAPHA®; · · · · represents model-simulated matrine concentrations of rats receiving 0.38 g/kg ACAPHA®.

Figure 4. Time course of matrine plasma concentrations in rats receive (a) a bolus i.v. injection of 40 mg/kg pure matrine or (b) a single oral dose of 40 mg/kg pure matrine. Solid line represents model simulation. Data points represent the mean of experimental data. They were read digitally from the publication of Wu et al. (2003).
**TABLES**

Table 1. Parameters used in the physiologically based pharmacokinetic model of matrine for the rat*

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Blood flow rates (Percentage of cardiac output**)</th>
<th>Volume (Percentage of body weight)</th>
<th>Tissue:plasma partition coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>7.0</td>
<td>7.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Brain</td>
<td>2.0</td>
<td>0.57</td>
<td>2.0</td>
</tr>
<tr>
<td>Heart</td>
<td>4.9</td>
<td>0.33</td>
<td>1.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>14.1</td>
<td>0.73</td>
<td>10</td>
</tr>
<tr>
<td>Liver</td>
<td>17.5</td>
<td>3.66</td>
<td>5.5</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>13.1</td>
<td>2.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Muscle</td>
<td>27.8</td>
<td>40.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.0</td>
<td>0.2</td>
<td>6.3</td>
</tr>
<tr>
<td>Blood</td>
<td>-</td>
<td>8.2</td>
<td>-</td>
</tr>
<tr>
<td>Rest of the body</td>
<td>26.7</td>
<td>35.1</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*Mean data on tissue blood flow rate and volume were adapted from Luttringer et al., (2003).

**Cardiac output was scaled from an allometric equation (14.1 x body weight\(^{0.75}\)) based on 7.08 L/hr for a 0.4 kg rat.
Table 2. Pharmacokinetic parameters of rats receive a single oral dose of pure matrine or ACAPHA®

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Pure matrine</th>
<th>ACAPHA®</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLPLR</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>$k_a$ (hr⁻¹)</td>
<td>6.5</td>
<td>4.0 (4.0)**</td>
</tr>
<tr>
<td>EH</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>F</td>
<td>0.85</td>
<td>0.35 (0.85)**</td>
</tr>
<tr>
<td>t_{lag} (hr)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>$k_f$ (hr⁻¹)</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>CL (L/hr-kg)</td>
<td>1.2</td>
<td>0.20 (0.35)**</td>
</tr>
</tbody>
</table>

* Rats were treated with a single oral dose of pure matrine (15 mg/kg) or ACAPHA® (3.8 g/kg or 0.38 g/kg). BLPLR, the blood/plasma ratio of matrine; EH, hepatic extraction ratio; $k_a$, the absorption rate constant; F, the experimental bioavailability factor; t_{lag}, the lag time for absorption; $k_f$, fecal excretion rate constant; CL, the renal clearance of matrine.

** These parameters were optimized based on the experimental data of the ACAPHA® studies; parameter values in brackets were for the 0.38 g/kg ACAPHA® study.
Table 3. Mean prediction errors over the entire simulation time ranges

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pure matrine (15 mg/kg)</th>
<th>ACAPHA* (0.38 g/kg)</th>
<th>ACAPHA* (3.8 g/kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>-19.0</td>
<td>-42.4</td>
<td>-14.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>-0.7</td>
<td>-39.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>-59.9**</td>
<td>-13.9</td>
<td>-11.7</td>
</tr>
<tr>
<td>Liver</td>
<td>-34.6</td>
<td>-33.8</td>
<td>-25.5</td>
</tr>
<tr>
<td>Brain</td>
<td>-28.6</td>
<td>-10.8</td>
<td>-31.6</td>
</tr>
<tr>
<td>Heart</td>
<td>-36.6</td>
<td>30.4</td>
<td>-45.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>-1.5</td>
<td>16.5</td>
<td>-21.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>-7.2</td>
<td>8.7</td>
<td>26.9</td>
</tr>
<tr>
<td>Lung</td>
<td>-57.0**</td>
<td>-35.2</td>
<td>-14.9</td>
</tr>
</tbody>
</table>

* Matrine equivalent dose was 15 mg/kg
**Mean prediction error absolute value >50%
Table 4. Normalized sensitivity coefficients for matrine concentrations in the lung and the plasma

*See Table 2 for explanation on abbreviations of the parameters

** Numbers without brackets are sensitivity coefficients of the lung; numbers in brackets are sensitivity coefficients of the blood

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Post-dosing time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>High (&gt;0.5)</strong></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>-0.93</td>
</tr>
<tr>
<td></td>
<td>(-0.62)**</td>
</tr>
<tr>
<td>BLPLR</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>(-0.86)</td>
</tr>
<tr>
<td>F</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>(0.86)</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td></td>
</tr>
<tr>
<td>kₐ</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>(-0.09)</td>
</tr>
<tr>
<td><strong>Low (&lt;0.05)</strong></td>
<td></td>
</tr>
<tr>
<td>EH</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>(-0.01)</td>
</tr>
<tr>
<td>tlag</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
</tr>
<tr>
<td>kᵣ</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>(0.00)</td>
</tr>
</tbody>
</table>
Figure 1

![Chemical structure diagram](image-url)
Figure 3

(a) plasma

Matrine conc. in plasma (ng/ml)

Log scale

Post-dosing time (hr)

0 2 4 6 8 10 12

10^0 10^1 10^2 10^3 10^4 10^5
Figure 3

(c) spleen

Matrine conc. in spleen (ng/g)

Post-dosing time (hr)

0 2 4 6 8 10 12

$10^1$ $10^2$ $10^3$ $10^4$ $10^5$
Figure 3

(d) brain

Matrine conc. in brain (ng/g)

Post-dosing time (hr)

10^5

10^4

10^3

10^2

10^1

0  2  4  6  8  10  12
Figure 3

(f) muscle

Matrine conc. in muscle (ng/g)

Post-dosing time (hr)

Log scale for concentration and time.
Figure 3

(g) lung

Matrine conc. in lung (ng/g)

Post-dosing time (hr)
Figure 3

(h) liver

Matrine conc. in liver (ng/g)

Post-dosing time (hr)
Figure 3

(i) fat

Matrine conc. in fat (ng/g)

Post-dosing time (hr)

0 2 4 6 8 10 12

$10^0$ $10^1$ $10^2$ $10^3$ $10^4$
Figure 4a

Matrine conc. in plasma (ng/ml)

Post-dosing time (hr)

10^5

10^4

10^3
Figure 4b
APPENDIX

Mass balance equations for the PBPK model depicted in Figure 2 describing matrine concentrations in arterial blood (C_BA), venous blood (C_BV), lung (C_LG), heart (C_HR), kidney (C_KD), fat (C_FT), muscle (C_MS), brain (C_BR), liver (C_LV), spleen (C_SP), gut tissue (C_GT), gut contents (C_GC) and the rest of body (C_RB) are presented.

Non-eliminating organs and tissues:

\[ V_{XY}(dC_{XY}/dt) = Q_{XY}(C_{BA} - C_{XY}/P_{XY}) \]  
(1)

where, \( XY \) represents the heart, brain, spleen, fat, muscle, gut, and the rest of body. The terms \( Q_{XY}, V_{XY} \) and \( P_{XY} \) represent tissue blood flow, volume and tissue/blood partition coefficient, respectively.

Liver:

\[ V_{LV} (dC_{LV}/dt) = ((Q_{LV} - Q_{GU} - Q_{SP})C_{BA} + Q_{GU}(C_{GU}/P_{GU}) + Q_{SP}(C_{SP}/P_{SP})) - Q_{LV}(C_{LV}/P_{LV} - R) \]  
(2)

\[ R = ((Q_{LV} - Q_{GU} - Q_{SP})C_{BA} + Q_{GU}(C_{GU}/P_{GU}) + Q_{SP}(C_{SP}/P_{SP}))EH \]

where EH is hepatic extraction ratio. \( R \) represents biliary excretion of matrine.

Gut tissue:

\[ V_{GT} (dC_{GT}/dt) = (Q_{GT} (C_{BA}-C_{GT}/P_{GT}) + R_{ao}) \]  
(3)

\[ R_{ao} = (k_a)(F)(dose)exp^{-k_a(t-tlag)} \]

where, \( R_{ao} \) is matrine input rate into the blood from the gut; \( k_a \) is the absorption rate constant of matrine; dose represents matrine equivalents in ACAPHA® or pure matrine; \( F \) is the apparent or empirical bioavailability factor; \( tlag \) is the lag times for absorption.

Gut contents:

\[ V_{GC} (dC_{GC}/dt) = (R - k_f(C_{GC})(V_{GT})) \]  
(4)

where \( k_f \) is the fecal excretion rate constant
Kidney:

\[ V_{KD} \frac{dC_{KD}}{dt} = (Q_K(C_{BA}-C_{KD}/P_{KD})-CL(C_{BA})) \]  

where CL is the renal clearance of matrine.

Venous blood:

\[ V_{BV}(dC_{BV}/dt) = (Q_{FT}(C_{FT}/P_{FT}) + Q_{HR}(C_{HR}/P_{HR}) + Q_{KD}(C_{KD}/P_{KD}) + Q_{MS}(C_{MS}/P_{MS}) + Q_{LV}(C_{LV}/P_{LV}) + Q_{BR}(C_{BR}/P_{BR}) + Q_{RB}(C_{RB}/P_{RB}) - (QC)C_{BV}) \]  

Arterial blood:

\[ V_{BA}(dC_{BA}/dt) = QC(C_{LG}/P_{LG} - C_{BA}) \]  

Lung:

\[ V_{LG}(dC_{LG}/dt) = QC(C_{BV} - C_{LG}/P_{LG}) \]