Metabolite Kinetics: The Segregated Flow Model for Intestinal and Whole Body Physiologically Based Pharmacokinetic Modeling to Describe Intestinal and Hepatic Glucuronidation of Morphine in Rats In Vivo

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ABSTRACT

We used the intestinal segregated flow model (SFM) versus the traditional model (TM), nested within physiologically based pharmacokinetic (PBPK) models, to describe the biliary and urinary excretion of morphine 3β-glucuronide (MG) after intravenous and intraduodenal dosing of morphine in rats in vivo. The SFM model describes a partial (5%–30%) intestinal blood flow perfusing the transporter- and enzyme-rich enterocyte region, whereas the TM describes 100% flow perfusing the intestine as a whole. For the SFM, drugs entering from the circulation are expected to be metabolized to lesser extents by the intestine due to the segregated flow, reflecting the phenomenon of shunting and route-dependent intestinal metabolism. The poor permeability of MG crossing the liver reflects the phenomenon of shunting and route-dependent intestinal metabolism. The poor permeability of MG across the liver or intestinal basolateral membranes mandates that most of MG that is excreted into bile is hepatically formed, whereas MG that is excreted into urine originates from both intestine and liver metabolism, since MG is effluxed back to blood. The ratio of MG amounts in urine/bile for intraduodenal/intravenous dosing is expected to exceed unity for the SFM but approximates unity for the TM. Compartmental analysis of morphine and MG data, without consideration of the permeability of MG and where MG is formed, suggests the ratio to be 1 and failed to describe the kinetics of MG.

The observed intraduodenal/intravenous ratio of $\frac{A_{\text{MG, urine, 4h}}}{A_{\text{MG, bile, 4h}}}$ (2.55 at 4 hours) was better predicted by the SFM-PBPK (2.59 at 4 hours) and not the TM-PBPK (1.0), supporting the view that the SFM is superior for the description of intestinal-liver metabolism of morphine to MG. The SFM-PBPK model predicts an appreciable contribution of the intestine to first pass M metabolism.

Introduction

It has been recognized that, generally, compartmental modeling is unable to quantitatively address the multiplicity of metabolite formation organs and does not account for sequential metabolism/excretion nor permeability barriers of formed metabolites (Pang et al., 2008; Pang, 2009). In contrast, physiologically based pharmacokinetic (PBPK) models address events of sequential elimination and include transmembrane barriers (de Lannoy and Pang, 1986, 1993; Pang, 2003; Pang et al., 2009; Chow and Pang, 2013) and transporters (Sun et al., 2006, 2010). The intestine, richly endowed with enzymes and transporters (van Herwaarden et al., 2007; Zhang et al., 2007, 2009; Liu et al., 2010), strongly affects first-pass metabolism and controls the flow of substrate to the liver (Pang and Chow, 2012). Intestinally formed metabolites may undergo immediate sequential metabolism or excretion (Pang and Gillette, 1979). When the metabolite possesses good permeability or transporter-linked properties, it will cross the liver cell membrane to endure liver metabolism and/or biliary excretion prior to reaching the lung, heart, and general circulation.

Route-dependent metabolism by the intestine is repeatedly being observed (namely, a higher extent of intestinal metabolism exists when a drug is given orally versus the lower extent or virtual absence of intestine metabolism when the drug is given systemically) (Cong et al., 2000; Doherty and Pang, 2000; Fan et al., 2010). This was observed for erythromycin (Lown et al., 1995) and midazolam (Paine et al., 1996) in humans and for enalapril hydrolysis (Pang et al., 1985) and morphine glucuronidation in the rat intestine (Doherty and Pang, 2000). The lesser extent of intestinal metabolism for systemically delivered drugs is explained by the pattern that a fraction and not the entire intestinal blood flow perfuses and recruits enzymes/excretory transporters in the enterocyte region, with the majority of flow perfusing the inactive, serosal region (Cong et al., 2000; Fan et al., 2010). These observations led to the development of the segregated flow model (SFM), describing that only a partial intestinal flow (5%–30%) reaches the enterocyte region to explain the higher oral versus intravenous intestinal metabolism. In contrast, the traditional model (TM) describes no difference, when the entire intestinal flow perfuses the intestinal tissue as a whole (Cong et al., 2000).

In this study, we examined morphine glucuronidation in the rat in vivo after administration of small doses of natural (−)-morphine (M) in saline...
into the jugular vein for intravenous or duodenal lumen [or intra-
duodenal (ID)] dosing, with continuous bile collection via a catheter. We
studied M, which enters the cell by passive diffusion (Doherty et al.,
2006) and is primarily glucurononated at the 3-position to form morphine
3β-glucuronide (MG) by the rat UDP-glucuronosyltransferase 2 family,
polypeptide b1, Ugt2b1, in the rat intestine and liver (Rane et al., 1985).
Morphine is also known to be metabolized by cytochrome P450 (Projean
et al., 2003) and undergoes excretion via P-glycoprotein (Böerner 1975;
Iwamoto and Klaassen, 1977; Letrent et al., 1999; Wandel et al., 2002)
to minor extents. The rat kidney actively secretes but does not me-
tabolize M (Van Crugten et al., 1991; Shanahan et al., 1997). MG is
excreted from formation tissues; enterobiliary circulation in rats has
been noted (Dahlström and Paalzow, 1978; Horton and Pollack, 1991)
but not for the rat with an open bile fistula. The influx permeability
clearance of MG through the liver (0.1 ml min⁻¹ g⁻¹ liver) was
estimated to be 5%–10% of the flow rate, suggesting the existence of a
diffusional barrier for MG to enter the hepatocyte (Doherty et al.,
2006). Intestinally formed MG undergoes luminal secretion via the mul-
tiple drug resistance-associated protein 2 (Mrp2) and is effluxed into
the circulation via the multiple drug resistance-associated protein 3 (Mrp3),
in the rat (van de Wetering et al., 2007). MG formed in the liver is
biliarily excreted as well as effluxed out. These MG species of intestinal
and hepatic origins that reenter the circulation are excreted by the kid-
ney, with clearance (CL) values that are similar to the glomerular
filtration rate (Van Crugten et al., 1991). Intuitively speaking, the extents
of intestine versus liver formation of MG, reflected by their appearance
in urine/bile, should remain the same for both intravenous and
intraduodenal dosing, when the flow patterns for the delivery of M to
the intestine and liver are the same for different routes of drug admin-
istration, as with the TM model. By contrast, when M in the systemic
circulation is being partially shunted away from the enterocyte for
metabolism with intravenous dosing for the SFM model, the urine/bile
ratio of MG for intravenous dosing of M is expected to be lower than that
for intraduodenal dosing. The different extents of excretion of MG in
bile versus urine for intraduodenal versus intravenous dosing of M could
be used to appraise which intestine model, TM or SFM, best describes
first-pass metabolism when nested within PBPK models.

Materials and Methods

Materials

M and MG were provided by the National Institutes of Health National Institute
on Drug Abuse (Rockville, MD); caffeine, the internal standard (IS), was
purchased from Sigma-Aldrich Co. (St. Louis, MO). High-performance liquid
chromatography (LC)–grade acetonitrile, methanol, and formic acid were
obtained from Fisher Scientific Canada (Ontario, ON, Canada). Male Sprague-
Dawley rats (St. Constant, QC, Canada), weighing 305 ± 10 g (aged 8 to
9 weeks), were used throughout the study.

In Vivo Pharmacokinetic Study

Rats were maintained under constant housing and environmental condi-
tions (temperature, lighting, and diet) according to protocols approved by
the University of Toronto. Rats were anesthetized from food but were given 5% (v/v)
glucose water overnight before the day of the study. Pentobarbital (65 mg kg⁻¹,
given intraperitoneally) was used to induce anesthesia, since ketamine was
given intraperitoneally) was used to induce anesthesia, since ketamine was
previously reported to inhibit morphine glucuronidation (Qi et al., 2010). Under
anesthesia, the carotid artery was cannulated with PE50 tubing, which was
prefilled with heparinized (1000 U/ml) physiologic saline solution for sampling;
the contralateral jugular vein was cannulated for the intravenous administration of M (Hirayama et al., 1990). The intraduodenal dose solution was introduced as
a bolus needle injection into the proximal duodenum. A midline incision was
made for bile duct cannulation with PE50 tubing. The opened neck and abdominal
regions for the surgical manipulations were sutured immediately after drug
administration. For intravenous administration, M (expressed as morphine base,
14.9±2.6 μmol·kg⁻¹ in 0.2 ml saline solution) was administered as a bolus into
the jugular vein, followed by flushing of the inline contents with saline. For
intraduodenal administration, morphine sulfate (expressed as M, 26.6 ± 0.40
μmol·kg⁻¹ in 0.3 ml saline solution) was injected into the proximal duodenal lumen.
The difference in weights of the syringe before and after the injection was taken as
the volume of dose injected, and the dose solution was assayed by LC/mass
spectrometry. Blood (0.1 ml) was collected via the carotid artery cannula of the same
rat at 0, 1, 5, 10, 15, 30, 45, 60, 90, 120, and 180, and 240 minutes after dosing for each
rat. Bile was collected in toto via the bile duct cannula at 0, 5, 10, 15, 20, 30, 45, 60,
90, 180, and 240 minutes after dosing into prepared 1.5-ml vials. At the end of study
(240 minutes), the entire urinary content was collected from the bladder via sampling
with a needle/syringe. All samples were kept frozen at −20°C until analysis.

LC/Mass Spectrometry Assay

Protein Precipitation and Solid Phase Extraction. A set of standards of
known, added amounts of M and MG in blood was processed in the same manner
as the samples. Caffeine (IS; 10 μl of a 5 μg·ml⁻¹ solution) was added to 100 μl blood,
followed by protein precipitation with 400 μl of an equimixture of methanol
and acetonitrile, which was found to yield the highest recovery of the compounds.
After vortex mixing for 60 seconds and centrifugation at 13,000g for 10 minutes,
the supernatant was transferred into Sep-Pak Vac C18 3-cc cartridges (200 mg:
Waters, Milford, MA). Each cartridge was preconditioned with 2 × 1 ml acetonitrile
followed by 2 × 1 ml MilliPore water (Millipore, Billerica, MA). After loading of sample, 0.5 ml 5% acetonitrile in water was added into the cartridge and the contents in the cartridge were eluted with 2 × 1 ml acetonitrile. The eluent was pooled and dried under N₂ at room temperature. The residue was
reconstituted with 200 μl mobile phase [70% water with 0.1% (v/v) formic acid
and 30% acetonitrile with 0.1% (v/v) formic acid], and 5 μl reconstituted sample
was injected into the LC-MS/MS system.

Calibration curves for the quantification of M and MG in bile and urine
samples were constructed under identical conditions. Because of the differential
amounts of MG and M in bile, 10 μl bile (for MG assay) and 40 μl bile (for
morphine assay) was assayed in separate runs. Samples were spiked with 5 or
10 μl IS solution, then diluted with saline to 100 μl, before mixing with 400 μl
methanol and acetonitrile [1:1 (v/v)] for solid phase extraction loading. For urine
analysis, 10 μl of the urine sample was spiked with 10 μl IS solution and diluted to
100 μl with saline, then mixed with 400 μl methanol and acetonitrile [1:1 (v/v)],
for solid phase extraction loading. These samples were then processed similarly
to that described for the blood samples.

LC-MS/MS. LC-MS/MS was composed of an Agilent 1200 series liquid
chromatograph coupled to an Agilent 6410 triple-quadrupole mass spectrometer
with an electrospray source (Agilent Technologies, Santa Clara, CA). A high-
performance LC gradient consisting of the mobile phase components of 0.1%
formic acid in water (A) and 0.1% formic acid in acetonitrile (B), increasing from
4% to 30% between 5 and 10 minutes, then returning to 4% over the next minute,
was developed to separate MG, morphine, and caffeine (IS) at retention times
of 2.6, 4.3, and 10.9 minutes, respectively. Transitions from precursor ion
to product ion were observed with multiple reaction monitoring, as shown with the
relative mass-to-charge ratio (m/z): MG (m/z 462 → 286), morphine (m/z
286.1 → 165), and caffeine (m/z 195 → 138). Values for fractions or volume
collection energy were 160 V and 32 V for MG, 165 V and 40 V for M, and
85 V and 24 V for caffeine, respectively. The area of each peak, obtained by
MassHunter work station software (Agilent Technologies), was normalized to that
of the IS. A good correlation that showed linearity of (R² > 0.997) between
the added compound/IS area ratio versus the amount of compound in the sample
(blood/bile/urine) was observed. The coefficient of variation (CV) was < 14% for all
of the concentrations studied. The intraday CV was between 0.4% and 9.2%
for M concentrations ranging between 20 and 2470 ng·ml⁻¹ and between 0.9%
and 12.9% for MG for concentrations ranging between 16 and 2390 ng·ml⁻¹.
The data showed good linearity for the blood (R² > 0.997), urine (R² > 0.98), and bile
(R² > 0.98) calibration curves, and the limit of quantification was 9.75 and
19.5 ng ml⁻¹ for morphine and MG, respectively.

Data Analysis

Noncompartmental/Compartmental Analyses. The total area under the
concentration-time curve (AUC) to infinity was estimated as the sum of AUC₀₋₉₀,
obtained with the trapezoidal rule, and AUC₉₀₋∞ obtained by dividing the blood
concentration of the last sampling point (\(C_{\text{last}}\)) by \(b\), the terminal slope. The total body (blood) clearance (\(CL_{\text{tot}}\)) was calculated as \(\text{Dose}_{\text{IV}}/\text{AUC}_{\text{iv}}\). The bioavailability (or \(F_{\text{sys}}\)) was calculated from the dose-normalized \(\text{AUC}_{\text{IV}}/\text{AUC}_{\text{iv}}\) or approximated by the amounts of M excreted into urine at 4 hours after intraduodenal/intravenous dosing. Concentration and amount data were normalized to dose, and data were expressed means ± S.D.

A two-compartment model for M and a one-compartment model for MG were used for compartmental analysis and fitting of M and MG data (Fig. 1). The total elimination rate constant of M arising from the central compartment (\(k_3\)) comprises the metabolic (\(k_b\)), biliary (\(k_{\text{biliary}}\)), and renal (\(k_{\text{renal}}\)) excretion rate constants and \(k_{\text{other}}\), for other metabolic pathways; \(k_1\) and \(k_2\) denote the absorption and intercompartmental rate constants, respectively; and \(V_1\) and \(F_{\text{abs}}\) are the central volume of distribution and fraction of dose absorbed, respectively. The metabolite, MG, with volume of distribution \(V\), is excreted into bile and urine, with rate constants \(k_{\text{m[LB]}}\) and \(k_{\text{m[renal]}}\) respectively.

**PBPK Models.** The TM-PBPK and SFM-PBPK models (Fig. 2) were used for optimization of the intravenous and intraduodenal blood, bile and urine data of M and MG. Five tissues were considered: rapidly perfused (RP) tissue, poorly perfused (PP) tissue, and adipose (AD) tissue, liver (L), and intestine (I), which are denoted as subscripts and interconnected by blood flow (\(Q\)). For detailed consideration of first-pass metabolism of M, the liver and intestinal tissues were subcompartmentalized as the tissue and tissue blood compartments to better accommodate the permeability barrier of M. In intestinal blood (IB) and liver blood (LB) rapidly exchanges with those in tissue with influx (\(CL_{\text{inf},M}\) and \(CL_{\text{inf},L}\), high values) and efflux (\(CL_{\text{eff},M}\) and \(CL_{\text{eff},I}\), high values) clearances, respectively. In liver, M forms MG and other metabolites with intrinsic clearances \(CL_{\text{M→MG}}\) and \(CL_{\text{M→other}}\), respectively, or it is biliary excreted (\(CL_{\text{M→biliary}}\)). The MG formed in the intestine and liver is either effluxed out with \(CL_{\text{MG→for}}\) secreted into the intestinal lumen and bile canaliculi with secretory intrinsic clearances \(CL_{\text{MG→int}}\) and \(CL_{\text{MG→L}}\) respectively. MG, M and MG in intestinal blood (IB) and liver blood (LB) rapidly exchanges with those in tissue with influx (\(CL_{\text{inf},M}\) and \(CL_{\text{inf},L}\), high values) and efflux (\(CL_{\text{eff},M}\) and \(CL_{\text{eff},I}\), high values) clearances, respectively. The SFM, the intestine is further subdivided into the enteroce (en) and serosal (s) regions and the corresponding blood regions (enB and sB); the enteroce flow, \(Q_{\text{en}}\), perfusing the metabolically active and transporter-rich region is only a small fraction (\(q_0 = 0.05\) to 0.3) of the total intestinal flow rate, \(Q_1\) or \(Q_2\), the serosal flow, \(Q_2\), is \((1 - q_0)\) \(Q_1\) (Fig. 2B) (Cong et al., 2000).

**Fitting.** Fitting was conducted by ADAPT 5 Systems Analysis Software (BMSR Biomedical Simulations Resource, Version 5; University of Southern California, Los Angeles, CA). The population method and the maximum likelihood with the Expectation-Maximization (EM) algorithm were used to fit individual sets of data (intravenous, \(n = 4\); intraduodenal, \(n = 3\)) and the population data set, based on individual data sets. We employed a two-compartment model for M and a one-compartment model for the metabolite, MG, to fit individual data sets (data for each rat) and all of the data as a whole (Fig. 1). Fitted results furnished estimates of \(k_b\) total elimination (\(k_b\)), \(k_{\text{biliary}}\), \(k_{\text{renal}}\), \(k_{\text{other}}\), \(k_{\text{abs}}, k_1, k_2, V_1\), and \(F_{\text{abs}}\) with rate equations shown in Appendix A.

Then the TM-PBPK and SFM-PBPK models (Fig. 2) were used for fitting, with assigned physiologic volumes and flows that are obtained from literature values and summarized in Table 1. The mass balance equations for the TM-PBPK and SFM-PBPK models appear in Appendix B. The transport clearances for M (\(CL_{\text{M\rightarrowL}}\), \(CL_{\text{M\rightarrowI}}\), \(CL_{\text{M\rightarrowbiliary}}\), \(CL_{\text{M\rightarrowother}}\), and \(CL_{\text{M\rightarrowfor}}\)) were first assigned as 5 × flow to tissue; the tissue to blood partitioning coefficients of M for the rapidly perfused tissue (\(K_{\text{RPP}}\)), poorly perfused tissue (\(K_{\text{PFP}}\), and adipose tissue (\(K_{\text{AFP}}\)), calculated according to Rodgers and Rowland (2006, 2007), were used as initial estimates (Table 2) and the parameters were optimized by fitting. Similar \(K_{\text{f}}\) values for MG were not needed since transport terms were used for the intestine and liver, the few tissues where MG was distributed. The equations, assumptions, and mass balance equations are shown in Appendix B. Only the unbound species was involved in transport and elimination; the unbound fraction in plasma (\(f_0\)) was corrected by the blood/plasma concentration ratio \(C_{\text{B/P}}\) to obtain the unbound fraction in blood, \(f_B\). The tissue unbound fraction (\(f_2\)) and the intrinsic metabolic or transport clearance were estimated as a combined parameter. All of the intrinsic clearances for metabolism (\(CL_{\text{inf,m}}\)) and secretion (\(CL_{\text{inf,s}}\)) for the intestine (I) and liver (L), as well as the rate constants for absorption (\(k_a\)), and fraction of dose absorbed in gut lumen (\(F_{\text{m[LB]}}\)), were obtained by fitting. \(F_{\text{abs}}\) is the ratio of \(k_b/(k_b+k_h)\) where \(k_h\) is the luminal degradation rate constant.

We also fitted the data with the nested TM- and SFM-PBPK models. With the fitted constants, simulations were extended to time infinity to estimate the amounts of MG in bile and urine for the TM- or SFM-intestine compartment nested in the PBPK model. Ratios of amounts of MG excreted into urine and bile after intraduodenal and intravenous dosing of M were then compared for the TM and SFM-PBPK models. The final model was selected based on the goodness-of-fit criteria, which included convergence, parameter precision, and visual inspection of predicted versus observed values and residual plots. The sum of squared residuals, CV (or standard deviation of fitted parameter/parameter estimate), residual plots, as well as the \(F\) test were used to compare goodness of fit of the nested TM- and SFM-PBPK models (Boxenbaum et al., 1974).

**Mass Balance Solutions for M and MG Amounts in Bile and Urine.** Simple mass balance considerations were developed to illustrate the relationship between the intestine and liver in forming the metabolite in question. It was assumed that the intestine and liver are the only two organs capable of forming MG, and M is completely absorbed. For simplification, MG is assumed as unable to enter the intestine or liver. Mass balance relations involving the intestinal and hepatic availabilities/extraction ratios of M and MG, the formed metabolite, are included to describe the formation of MG by the intestine and liver and in the sequential removal of MG.

**Statistical Comparisons**

The two-tailed \(t\) test was used to compare the means, and a \(P\) value of <0.05 was viewed as significant.

**Results**

In Vivo Pharmacokinetics of M after Intravenous and Intraduodenal Dosing to Rats: Noncompartmental Analysis

M decayed biexponentially after intravenous dosing, although the biphasic profile was not apparent after intraduodenal dosing (Fig. 3). The terminal half-lives for M, estimated by regression of log-linear portion of decay curves, were identical for intraduodenal and intravenous dosing (61 and 67 minutes; \(P > 0.05\)). The area under the blood concentration-time curve for M (\(\text{AUC}_{\text{IV}}\)), obtained by summing the AUC by the trapezoidal rule and extrapolated area (\(\text{AUC}_{\text{inf}}\)), yielded a total body blood clearance (\(CL_{\text{B}}\)), of 6.6 ± 3.3 ml min\(^{-1}\), a value comparable to the blood clearance of 6.31 ml min\(^{-1}\) (\(CL_{\text{B}}/CL_{\text{P}}\)) according to Mistry and Houston (1987), based on plasma clearance, \(CL_{\text{P}}\), of 8.46 and a \(CL_{\text{P}}/CL_{\text{B}}\) ratio of 1.34). Both M and MG were recovered in bile and urine in different proportions (Fig. 3; Table 3). The renal clearance of M,
AUC<sub>MG</sub> ratio for intraduodenal/intravenous dosing would not reflect the systemic availability. The renal clearance of MG, approximated by \( \frac{A_{\text{MG urine,4h,IV}}}{\text{AUC}_{\text{MG,4h,IV}}} \), was 2.63 ml·min<sup>-1</sup>, and it was 2.68 ml·min<sup>-1</sup> after correction for the unbound fraction in plasma (0.98); the value is slightly lower than the glomerular filtration rate of Marcel de Vries et al. (1997). The percent dose of MG in bile at 4 hours (\( \frac{A_{\text{MG urine,4h,IV}}}{\text{Dose}} \)) was 80% higher for intraduodenal dosing than with intravenous dosing, whereas the percent dose of MG recovered in urine at 4 hours (\( \frac{A_{\text{MG urine,4h,IV}}}{\text{Dose}} \)) for intraduodenal dosing was 4.65-fold that for the intravenous dose (Table 3). As a result, the AUC<sub>MG</sub> ratio was 2.55 times that of \( \frac{A_{\text{MG urine,4h,IV}}}{\text{AUC}_{\text{MG,4h,IV}}} \) at 4 hours after dosing (Table 3).

Compartmental Modeling of M and MG

Fitting of the blood concentration-time profiles of M and MG after intravenous and intraduodenal dosing was generally satisfactory for both routes of administration (Fig. 3). However, MG in bile was overestimated for intravenous data but underestimated for intraduodenal data, whereas MG in urine was overpredicted for intravenous dosing but underpredicted for intraduodenal dosing. The AUCs provided an estimate of 0.95 for \( \frac{F_{\text{mg}}}{10} \), that was higher than observed. The calculated, total clearance (\( k_{10}V_1 \)) was 0.0711 * 140 or 9.95 ml·min<sup>-1</sup> (Table 4) and was higher than that observed (Table 3). According to the ratio of each rate constant/\( k_{10} \), the pathways for formation of other metabolites (1.4%), biliary excretion (less than 1%), and renal excretion (31%) contributed much less to the total elimination compared with the glucuronidation pathway (67.5%) or \( k_{\text{ad}}/k_{10} \).

SFM-PBPK and TM-PBPK Modeling of M and MG

The tissue/blood concentration ratios, or the tissue partitioning ratios were calculated based on the methods of Rodgers and Rowland (2006, 2007) with use of known fractional volumes of the intracellular and extracellular tissue water, neutral lipid and phospholipid, and concentration of binding elements: extracellular albumin, acidic phospholipids, and neutral lipids and phospholipids; the pKa and the oil to water partition coefficient, \( P_{\text{ow}} \), for octanol/water and vegetable oil/water were used in the calculation. These were compared with the optimized tissue to blood partitioning coefficients (\( K_T \)) that were estimated by fitting (Table 2). Generally, the fitted estimates were within ±2-fold of the calculated values of \( K_{\text{PP}} \), \( K_{\text{pp}} \), and \( K_{\text{ad}} \).

The fits to the PBPK models were much improved compared with that from compartmental fitting (compare Figs. 3 and 4; Table 6). The blood levels of MG were less well predicted by the TM than for the SFM; MG appearance was overestimated in bile both after intravenous and intraduodenal dosing but was underestimated in urine after intraduodenal dosing by TM. Pictorially, predictions by the SFM-PBPK model provided data that closely matched the observed, temporal data for concentration, bile, and urinary profiles up to the 4 hours, compared with the TM (Fig. 4). The fitted parameters of the SFM-PBPK and TM-PBPK are summarized in Table 5. The predicted versus observed data (Figs. 5 and 6) showed that the SFM-PBPK model fitted the data better than the TM-PBPK model. The F test showed that the SFM-PBPK provided the best fits over those for the TM-PBPK and compartmental models (Table 6).

Additional parameters were obtained from PBPK modeling (Table 7). The apparent (unbound) tissue to blood partitioning ratios of M, obtained from the ratio \( \frac{f_{\text{t}} \cdot \text{CL}_{\text{t,ref}}}{\text{CL}_{\text{b,ref}}} \), and \( \frac{f_{\text{t}} \cdot \text{CL}_{\text{t,ref}}}{\text{CL}_{\text{b,ref}}} \) are 0.14 to 0.53 for the intestine.

Fig. 2. (A and B) TM-PBPK (A) and SFM-PBPK (B) models for describing the pharmacokinetics of M and MG. MG exhibits poor entry into tissues (including the intestine and liver) and MG is formed in the intestine and liver. Intestinally formed MG enters the liver with influx clearance (\( \text{Cl}_{\text{mg}} \)) of 1 ml·min<sup>-1</sup> (according to Doherty et al., 2006), and MG formed in the liver are excreted into bile. See the text for details on the definition of terms.
with both methods of estimation, with the latter calculated and 0.58 and 0.72 for the SFM, respectively (Table 7). The values were similar regardless of the equation used. For MG that is formed in tissue, the availability or fraction that escapes into the circulation, whereas $F_{\text{int}}$ or $F_{\text{eff}}$ was 0.12 to 0.22 for the liver for the TM- and SFM-PBPK models. The data showed little extraction of MG by the intestine but substantial extraction of MG by the liver, according to the TM- and SFM-PBPK models, respectively. The fraction of hepatic clearance of M forming MG, $h_{\text{int}}$, was obtained as the ratio of the formation intrinsic clearance/total intrinsic clearance, or $>85\%$ for both the TM- and SFM-PBPK models, showing that glucuronidation is a major elimination pathway in the liver. The fraction of total body clearance of M forming MG, $g_{\text{int}}$, was around $57\%–63\%$, a value similar to the estimate from the compartmental model. The value is lower since M is excreted unchanged into urine.

The fractional contributions of the intestine and liver to the first-pass removal were estimated. The extents of intestine and liver removal of M are highly dependent on $f_Q$, the fractional enteroocyte flow (Pang and Chow, 2012) (eqs. 1 and 2):
The percent contribution by the intestine was 46%–57% and 9.3%–17% for the TM-PBPK and SFM-PBPK models, respectively; the percent contribution by the liver was 43%–54% and 83%–91% for the TM-PBPK for SFM-PBPK models, respectively (Table 7). These values differed due to the two methods for estimating $F_I$ and $F_L$. The data

$$\frac{v_I}{v_I + v_L} = \frac{f_0 Q_I (1 - F_I)}{f_0 Q_I (1 - F_I) + E_L (Q_I \left(f_0 F_I + (1 - f_0)\right) + Q_{HA})}$$

$$\frac{v_L}{v_I + v_L} = \frac{E_L (Q_I \left(f_0 F_I + (1 - f_0)\right) + Q_{HA})}{f_0 Q_I (1 - F_I) + E_L (Q_I \left(f_0 F_I + (1 - f_0)\right) + Q_{HA})}$$
show that the SFM predicts a lesser contribution by the intestine for intestinal-liver removal of M when M in systemic circulation was presented to the intestine. The simulated $\frac{A_{\text{MG, b, IV}}}{A_{\text{MG, b, ID}}}$ and $\frac{A_{\text{MG, IV}}}{A_{\text{MG, ID}}}$ for the SFM-PBPK model were closer to the observations than those for the TM-PBPK (Table 8). These values were not changed dramatically upon extrapolation of the data to infinity.

**Mass Balance Solutions for TM-PBPK versus SFM-PBPK**

We also probed the mass balance relations for the TM versus the SFM. In this examination, several assumptions were made so that meaningful relations could be obtained easily: M is completely absorbed for the intraduodenal dose ($F_{\text{abs, ID}} = 1$) but there is no enterohepatic recirculation for M secreted back to the liver; M only forms MG and not other metabolites in the intestine and liver. These assumptions are quite reasonable in view of the fitted results (Tables 5 and 7). We further included renal excretion of M, with $f_d$ to define the fraction of the intravenous dose of M excreted unchanged. The most important assumption was that MG in the systemic circulation does not enter the intestine or liver but is renally excreted.

**TM-PBPK**

According to the TM-PBPK, the serial blood circuit delivering M and MG to the enterocyte (or whole intestine) region and the liver remains unchanged for both intravenous and intraduodenal dosing. The intestine exerts its strategic, anterior placement over the liver in its initial removal of substrates before the species reach the liver. The extent of MG formation by both the intestine and liver is given by $(E_I + F_I E_d)$. Thus, the percent contribution to MG formulation during the first pass by the intestine and liver is $\frac{E_I}{E_I + F_I E_L}$ and $\frac{F_I E_d}{E_I + F_I E_L}$, respectively. These fractions, when multiplied by the organ-appropriate available fractions for MG, $F_{\text{I,m}}$, and $F_{\text{I,L}}$ for the formed metabolite, yield the extents of formed MG entering the circulation $\left[\frac{E_I F_{\text{I,m}}}{E_I + F_I E_L} + \frac{F_I E_d F_{\text{I,L}}}{E_I + F_I E_L}\right]$. For the intestine and liver, the portions of the MG formed that are immediately excreted into the gut lumen and bile, respectively, are given by the extraction ratios $E_{\text{I,m}}$ and $E_{\text{I,L}}$. For intraduodenal and intravenous doses of M ($Dose_{\text{ID}}^M$ and $Dose_{\text{IV}}^M$), the amounts of MG in urine and bile for the TM are given by eqs. 3–6:

\[
A_{\text{MG, I, ID}} = E_I F_{\text{I,m}} + F_I E_d F_{\text{I,L}}
\]

\[
A_{\text{MG, I, IV}} = E_I F_{\text{I,m}} + F_I E_d E_{\text{I,L}}
\]

\[
A_{\text{MG, IV}} = (1 - f_d) Dose_{\text{IV}}^M \frac{E_I F_{\text{I,m}} + F_I E_d E_{\text{I,L}}}{E_I + F_I E_L}
\]

\[
A_{\text{MG, TM}} = (1 - f_d) Dose_{\text{IV}}^M \frac{E_I F_{\text{I,m}} + F_I E_d E_{\text{I,L}}}{E_I + F_I E_L}
\]

The ratios of the amounts of MG in urine/bile for intravenous and intraduodenal dosing of M are identical (eqs. 7–8):

**TABLE 3**

Noncompartmental data for M and MG for intravenous and intraduodenal data of morphine sulfate administration to the rat (305 ± 16 g).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous Dosing (n = 4)</th>
<th>Intraduodenal Dosing (n = 3)</th>
<th>Ratio of Intraduodenal/Intravenous Dosing</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M Dose ($\mu$mol kg$^{-1}$)</td>
<td>14.9 ± 1.6</td>
<td>26.6 ± 0.40</td>
<td>1.79</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Rat (g)</td>
<td>299 ± 6</td>
<td>307 ± 12</td>
<td>1.03</td>
<td>0.576</td>
</tr>
<tr>
<td>$AUC_{\text{M, IV}}^M$ (nM min·nmol dose$^{-1}$)</td>
<td>183 ± 98</td>
<td>39.9 ± 6.0</td>
<td>0.187</td>
<td>0.057</td>
</tr>
<tr>
<td>$AUC_{\text{M, IV}}^M$ (nM min·nmol dose$^{-1}$)</td>
<td>188 ± 97</td>
<td>43.2 ± 6.1</td>
<td>0.229*</td>
<td>0.053</td>
</tr>
<tr>
<td>$t_{1/2b}^M$ (min)</td>
<td>61 ± 12</td>
<td>67 ± 23</td>
<td>1.09</td>
<td>0.691</td>
</tr>
<tr>
<td>$CL_{\text{b, IV}}$ (ml min$^{-1}$)</td>
<td>6.57 ± 3.28</td>
<td>0.559 ± 0.095</td>
<td>0.568</td>
<td>0.221</td>
</tr>
<tr>
<td>$M_{\text{M, IV}}$ (% dose M excreted into bile at 4h)</td>
<td>0.984 ± 0.508</td>
<td>0.559 ± 0.095</td>
<td>0.568</td>
<td>0.221</td>
</tr>
<tr>
<td>$M_{\text{M, IV}}$ (% dose M excreted into urine at 4h)</td>
<td>33.4 ± 15.1</td>
<td>7.16 ± 2.68</td>
<td>0.215*</td>
<td>0.034*</td>
</tr>
<tr>
<td>Percent dose as M excreted into urine and bile (4h)</td>
<td>34.3 ± 15.4</td>
<td>7.17 ± 2.60</td>
<td>0.225</td>
<td>0.034*</td>
</tr>
<tr>
<td>$CL_{\text{b, IV}}$ or $A_{\text{MG, M, IV}}^M$ (ml min$^{-1}$)</td>
<td>2.21 ± 1.24</td>
<td>1.77 ± 0.45</td>
<td>0.800</td>
<td>0.589</td>
</tr>
<tr>
<td>MG $AUC_{\text{MG, IV}}^M$ (nM min·nmol dose$^{-1}$)</td>
<td>26.2 ± 6.8</td>
<td>53 ± 28</td>
<td>2.02</td>
<td>0.114</td>
</tr>
<tr>
<td>$AUC_{\text{MG, IV}}^M$ (nM min·nmol dose$^{-1}$)</td>
<td>30.3 ± 8.2</td>
<td>60.5 ± 29.0</td>
<td>2.0</td>
<td>0.10</td>
</tr>
<tr>
<td>$A_{\text{MG, IV}}^M$ (% dose of MG excreted into bile at 4h)</td>
<td>79 ± 11</td>
<td>72 ± 5</td>
<td>0.911</td>
<td>0.82</td>
</tr>
<tr>
<td>$A_{\text{MG, IV}}^M$ (% dose of MG excreted into urine at 4h)</td>
<td>32.8 ± 11.3</td>
<td>58.8 ± 6.1</td>
<td>1.79*</td>
<td>0.016*</td>
</tr>
<tr>
<td>$CL_{\text{MG, IV}}$ or $A_{\text{MG, M, IV}}^M$ (ml min$^{-1}$)</td>
<td>6.63 ± 3.1</td>
<td>30.7 ± 13.1</td>
<td>4.65</td>
<td>0.015*</td>
</tr>
<tr>
<td>Percent dose as MG into urine and bile (4h)</td>
<td>2.63 ± 11.9</td>
<td>6.40 ± 3.29</td>
<td>2.43</td>
<td>0.08</td>
</tr>
<tr>
<td>MG $A_{\text{MG, M, IV}}$</td>
<td>39.5 ± 12.7</td>
<td>89.5 ± 7.0</td>
<td>2.26</td>
<td>0.002*</td>
</tr>
<tr>
<td>$A_{\text{MG, IV}}$ (% dose MG into bile and 4h)</td>
<td>0.212 ± 0.078</td>
<td>0.541 ± 0.274</td>
<td>2.55</td>
<td>0.066</td>
</tr>
<tr>
<td>Percent dose – total recovery in bile and urinary (4h)</td>
<td>73.8 ± 11</td>
<td>97.2 ± 8.8</td>
<td>1.32</td>
<td>0.030*</td>
</tr>
</tbody>
</table>

* Doses of morphine sulfate, as morphine base equivalent.
\( ^{\text{a}} \)Summed MG and M amounts in urine and bile.
\( ^{\text{b}} \) $F_{\text{sys}}$ based on AUC ratio.
\( ^{\text{c}} \) $F_{\text{sys}}$ based on urinary data.

\( ^{*} P < 0.05, \) unpaired t test
TABLE 4
Fit to two-compartment model for M and MG (mi) after intravenous (14.9 ± 1.6 μmol·kg⁻¹) and intraduodenal (26.6 ± 0.40 μmol·kg⁻¹) doses of morphine sulfate to the rat (305 ± 16 g)

Fitted estimates are percent CV values expressed within parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Individual Fitted Value</th>
<th>Population Fitted Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$ (min⁻¹)</td>
<td>First-order transfer rate constant between central and peripheral compartments</td>
<td>0.404 (46.9)</td>
<td>0.363 (46.4)</td>
</tr>
<tr>
<td>$k_{21}$ (min⁻¹)</td>
<td>First-order transfer rate constant from central to peripheral compartment</td>
<td>0.143 (72.9)</td>
<td>0.113 (69.5)</td>
</tr>
<tr>
<td>$k_{01}$ (min⁻¹)</td>
<td>First-order elimination constant from central compartment</td>
<td>0.07111</td>
<td>0.0735</td>
</tr>
<tr>
<td>$k_{m, i}$ (min⁻¹)</td>
<td>First-order rate constant describing formation of MG from M</td>
<td>0.048 (24.3)</td>
<td>0.047 (20.9)</td>
</tr>
<tr>
<td>$k_{m, oth}$ (min⁻¹)</td>
<td>First-order rate constant describing formation of MG from other metabolites</td>
<td>0.001 (24.1)</td>
<td>0.001 (25.0)</td>
</tr>
<tr>
<td>$k_{bile}$ (min⁻¹)</td>
<td>First-order rate constant describing biliary secretion of M</td>
<td>0.0001 (31.0)</td>
<td>0.0004 (31.0)</td>
</tr>
<tr>
<td>$k_{renal}$ (min⁻¹)</td>
<td>First-order rate constant describing renal excretion of M</td>
<td>0.022 (26.8)</td>
<td>0.021 (23.6)</td>
</tr>
<tr>
<td>$V_{c}$ (ml)</td>
<td>Volume of distribution of central compartment</td>
<td>140 (59.1)</td>
<td>120 (58.0)</td>
</tr>
<tr>
<td>$k_{a}$ (min⁻¹)</td>
<td>First-order absorption rate constant of M</td>
<td>0.036 (37.3)</td>
<td>0.034 (35.2)</td>
</tr>
<tr>
<td>$C_{L_{u}}$ (ml·min⁻¹)</td>
<td>Total clearance of M</td>
<td>9.95</td>
<td>8.82</td>
</tr>
<tr>
<td>$e_{u}$</td>
<td>Fraction of total morphine clearance responsible for forming MG</td>
<td>0.675</td>
<td>0.642</td>
</tr>
<tr>
<td>$F_{ab}$</td>
<td>Fraction of dose absorbed</td>
<td>0.94 (30.0)</td>
<td>0.95 (24.8)</td>
</tr>
<tr>
<td>$k_{(mi)_{bile}}$ (min⁻¹)</td>
<td>First-order rate constant describing biliary excretion of MG</td>
<td>0.045 (59.1)</td>
<td>0.040 (46.4)</td>
</tr>
<tr>
<td>$k_{(mi)_{renal}}$ (min⁻¹)</td>
<td>First-order rate constant describing renal excretion of MG</td>
<td>0.020 (12.5)</td>
<td>0.020 (12.1)</td>
</tr>
<tr>
<td>$V_{m, others}$ (ml)</td>
<td>Volume of distribution of metabolite compartment</td>
<td>306 (36.0)</td>
<td>292 (29.8)</td>
</tr>
</tbody>
</table>

*Calculated based on fitted parameters: $k_{12} = k_{u} + k_{v1} + k_{bile} + k_{renal}$

SFM-PBPK

According to the SFM-PBPK model, MG is formed by the intestine and liver during the first-pass effect, but mostly from the liver upon recirculation due to the segregated flow pattern to the enterocyte region (Cong et al., 2000). With the assumption that circulating levels of M cannot reach the enterocyte region for intestinal metabolism, the amounts of MG detected into urine (AMG urine) and bile (AMG bile) according to the SFM for intraduodenal and intravenous dosing of M are given by eqs. 9–12:

$$\frac{AMG_{\text{urine,ID}}}{AMG_{\text{urine,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L + F_{12} [mi]_E$$

(7)

$$\frac{AMG_{\text{bile,ID}}}{AMG_{\text{bile,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L + F_{12} [mi]_E$$

(8)

In like fashion, it may be shown that

$$\frac{AMG_{\text{urine,ID}}}{AMG_{\text{urine,ID}}} = AMG_{\text{bile,ID}}$$

for the TM-PBPK model.

The ratios of the amounts are as follows (eq. 13 and 14):

$$\frac{AMG_{\text{bile,ID}}}{AMG_{\text{bile,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L$$

(13)

$$\frac{AMG_{\text{urine,ID}}}{AMG_{\text{urine,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L$$

(14)

Similarly, the ratios of MG amounts in bile and urine after the same doses of intraduodenal and intravenous dosing of M are as follows (eq. 15 and 16):

$$\frac{AMG_{\text{bile,ID}}}{AMG_{\text{bile,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L$$

(15)

$$\frac{AMG_{\text{urine,ID}}}{AMG_{\text{urine,ID}}} = E_1 F_{12} [mi]_E + F_{12} [mi]_L$$

(16)

From the above analyses, differences are expected to exist between the TM-PBPK and SFM-PBPK models. The identities $AMG_{\text{bile,ID}}$ and $AMG_{\text{urine,ID}}$ exist for the TM, and these relations are in stark contrast with those shown for the SFM, where $AMG_{\text{bile,ID}}> AMG_{\text{urine,ID}}$ in cases in which M from the circulation would enter the intestine via $f_{d}Q_{s}$, the true difference would fall in between unity (for the TM) and the theoretical SFM-PBPK estimate from the above example, since MG is able to enter the liver from the circulation and M is shunted away for metabolism by the intestine. These differences are exploited to discriminate between the SFM-PBPK and TM-PBPK models. It is further interesting to note that when there is complete absorption of M and absence of intestinal glucuronidation/secretion ($F_1 = 1$ and $E_1 = 0$), $AMG_{\text{bile,ID}} = AMG_{\text{urine,ID}}$, and $AMG_{\text{bile,ID}} = AMG_{\text{urine,ID}}$ for the TM-PBPK.
Fig. 4. (A and B) Observed blood concentration-time profiles of M and MG as well as the cumulative amounts of M and MG in bile and urine after intravenous (A) or intraduodenal (B) administration of M (IV, solid circles, n = 4; ID, open circles, n = 3; M and MG are denoted as red and blue symbols, respectively). Fitting was performed according to the TM or SFM models nested in PBPK models (TM-PBPK or SFM-PBPK). The fits of the model to blood concentrations of M and MG, as well as cumulative amounts of M and MG in bile and urine after intravenous and intraduodenal administration of M (SFM, solid line; TM, dashed line), are shown. Data are presented as means ± S.D. and are the same as those in Fig. 3. Note the improved correlation between predictions and observations for M and MG for the SFM and the less optimal fit of MG with the TM. ID, intraduodenal; IV, intravenous.
Discussion

With recognition that the intestine can significantly reduce the orally or intraduodenally absorbed dose during first-pass metabolism and that differential induction and inhibition patterns of the enzymes and transporters exist (see Pang and Chow, 2012; Chow and Pang, 2013), much effort is extended to separate the contributions of the intestine and liver in first-pass metabolism. The direct observations on intestinal metabolism could be deciphered for lorcainide metabolism in portacaval shunts in rodents (Gugler et al., 1975; Giacomini et al., 1980; Plänitz, et al., 1985) and midazolam oxidation in anhepatic patients after duodenal and intravenous administrations during transplant surgery (Paine et al., 1996). Others examined specific gene knockdown of Cyp3a and NADPH-cytochrome P450 reductase within the intestinal versus hepatic tissue to directly demonstrate the effect of the knockdown of intestinal versus liver enzymes in first-pass metabolism in vivo (van Herwaarden et al., 2007; Zhang et al., 2007, 2009). The method of comparison of plasma or blood AUCs of drug after oral, intraportal, and intravenous administration, supplemented by in vitro metabolic data, is commonly used to identify the presence of intestinal and extrahepatic versus liver drug metabolism (Iwamoto and Klaassen, 1977; Iwamoto et al., 1982; Cassidy and Houston, 1984, Mistry and Houston, 1987; Liu et al., 2010). Judging merely from the AUC of the blood concentration of the MG or formed metabolite, AUC_{MG}, it becomes difficult to tease out each of the individual contributions of the intestine and liver since multiple tissues are involved in the formation and sequential metabolism of the metabolite (Sun and Pang, 2010). The situation becomes more complex for metabolite kinetics when the metabolite formed undergoes sequential elimination (by metabolism or excretion) (Pang and Gillette, 1979), when a permeability barrier exists (de Lannoy and Pang, 1986), and when the intestine with segregated flow is involved for metabolite formation (Cong et al., 2000). The metabolism of M to MG by the intestine and liver in the formation organs exemplify this situation.

The inadequacy of the compartmental model is shown readily. The compartmental approach (Fig. 1) overpredicted MG excretion into bile
for intravenous dosing but underpredicted MG excretion for intraduodenal dosing of M; it also overpredicted MG excretion in urine for intravenous dosing, whereas it underpredicted MG excretion for intraduodenal dosing. The CL$_{tot}$ was 8.8 ml·min$^{-1}$ (Table 4), slightly overpredicting the observed CL$_{tot}$ (6.57 ml·min$^{-1}$); a higher $F_{abs}$ of approximately 0.95 (Table 4) versus that observed was obtained. Although the comparison of $k_m/k_{10}$ yielded the extent of MG formation (67.5%), other important parameters are unobtainable (compare Table 4 with Tables 5 and 7).

By contrast, we obtain much more insight on M and MG handling with TM- and SFM-PBPK modeling. The final model consists of uptake, transport, and metabolic pathways of M and MG (Table 5), when the liver (CL$_{MG_{L}}$ as 1 ml·min$^{-1}$) and intestinal (CL$_{MG_{I}}$ as 0 ml·min$^{-1}$) influx clearances for MG were assigned (Fig. 2), and sequential removal of MG is via secretion, in contrast with other metabolites that may undergo further metabolism (Pang and Gillette, 1979). We had tested other PBPK models (CL$_{MG_{L}}$ = 0 and CL$_{MG_{I}}$ > 0), but the fit did not improve. The final model revealed information on the effective partitioning ratio into tissue (0.14 and 0.53 for the intestine and 1.4 and 2.5 for the liver based on the TM-PBPK or SFM-PBPK model), and estimates of $h_{mi}$ and $g_{mi}$, the fractions of hepatic and total body clearance of M forming MG, respectively, with full accounting of the immediate excretion of the nascently formed MG, as $F_{mi}^{[mi]}_I$ and $F_{mi}^{[mi]}_L$ (Table 7). Moreover, the estimates of $F_I$ and $F_L$ that dissect the contribution of the intestine and liver first-pass removal were provided. We emphasize that there are differences in intestinal metabolism when M is entering the intestine from the circulation, and the SFM predicted a smaller intestinal contribution than the TM that during the recirculation of M (Table 7).

In pursuit of whether the SFM is superior to the TM in describing intestinal metabolism of morphine in vivo, we nested these intestinal models into the PBPK model for data fitting (Fig. 2). When both the intestine and liver are involved in formation of the metabolite, we illustrate that the metabolic data are best used to provide discrimination between the SFM-PBPK versus the TM-PBPK model. Therefore, we examined the metabolism of M and excretion of MG. M enters cells freely by passive diffusion, whereas the formed metabolite MG is poorly permeable across the intestine and liver basolateral membranes (Doherty...
et al., 2006; van de Wetering et al., 2007). MG formed in the intestine and liver is effluxed out by Mrp3 or excreted by Mrp2 into the lumen or bile, respectively. The MG in bile originates mostly from M metabolism and little MG formed from intestine is excreted into bile. In contrast, MG in urine originates from both the intestine and liver. Thus, after making some simple assumptions, differences are expected between the SFM-PBPK and TM-PBPK simulations, differences are expected between the SFM-PBPK and TM-PBPK.

The question that remains is why there is urgency to identify the proper intestinal model in PBPK modeling. Recent examination of intestinal flow models emphasized that the type of intestinal flow model chosen is important: TM, Q_int, gut model (Yang et al., 2007), or SFM, in which the fractional flow to enterocyte region (f_g) is 1, 0.484, and 0.1–0.3, respectively (Pang and Chow, 2012). For most substrates, the fitted f_g is < 0.2 (Pang and Chow, 2012; Chow and Pang, 2013), and it is 0.10 for this study (Table 5). Since the percent contribution of the intestine during recirculation of M is dependent on f_g (see equations shown as footnotes to Table 7), we expect the ranking of SFM < Q_int, gut model < TM to stand, whereas the opposite exists for the percent contribution of liver (SFM > Q_int, gut model > TM) (Pang and Chow, 2012). These interpretations could affect the translation of in vitro microsomal activity to the metabolic intrinsic clearance, C_{int,met} in vivo. The intestinal flow model chosen to represent the enterocyte flow may also influence values of F_{I} and E_{I}. The data of Mistry and Houston (1987) revealed a 24-fold microsomal activity ratio (C_{int,met} / C_{int,met,L}) in vitro; yet in vivo E_{I} and E_{L} values of 0.33 and 0.47, respectively, correlated with only a 37-fold intrinsic clearance ratio (calculated C_{int,met,L} / C_{int,met,L}) in vivo for morphine glucuronidation in the rat. Therefore, the flow pattern to the enterocyte region of the intestine may play a role in altering in vitro–in vivo extrapolation.

### Table 5

Fitted parameters for the PBPK models, with nested TM or SFM intestinal models, showing that SFM is the superior model

<table>
<thead>
<tr>
<th>Fitted Parameter</th>
<th>Definition</th>
<th>TM</th>
<th>SFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_Q )</td>
<td>Fraction of ( Q_t ) to enterocyte region</td>
<td>1</td>
<td>0.10 (11.7)</td>
</tr>
<tr>
<td>( k_a ) (min(^{-1}))</td>
<td>Absorption rate constant of morphine</td>
<td>0.03 (12.6)</td>
<td>0.028 (7.91)</td>
</tr>
<tr>
<td>( F_{\text{abs}} )</td>
<td>Fraction of dose absorbed in gut lumen</td>
<td>0.99 (10.2)</td>
<td>0.92 (10.7)</td>
</tr>
<tr>
<td>( f_{\text{in}}^{\text{CL,L,Gut}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net influx clearance of morphine in the intestine</td>
<td>0.821 (44.9)</td>
<td>1.35 (49.2)</td>
</tr>
<tr>
<td>( f_{\text{in}}^{\text{CL,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net influx clearance of M in liver</td>
<td>5.99 (34.9)</td>
<td>2.60 (39.9)</td>
</tr>
<tr>
<td>( f_{\text{in}}^{\text{CL,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net efflux clearance of M in liver</td>
<td>15.0 (43.8)</td>
<td>14.5 (17.6)</td>
</tr>
<tr>
<td>( f_{\text{out}}^{\text{CL,Gut}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net efflux clearance of M in intestine</td>
<td>10.6 (10.0)</td>
<td>5.90 (14.0)</td>
</tr>
<tr>
<td>( f_{\text{met}}^{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Metabolic intrinsic clearance of M forming MG in intestine</td>
<td>3.14 (34.2)</td>
<td>2.93 (22.9)</td>
</tr>
<tr>
<td>( f_{\text{met}}^{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net intestinal intrinsic secretion clearance for M</td>
<td>1.59 (9.92)</td>
<td>2.09 (11.1)</td>
</tr>
<tr>
<td>( f_{\text{met}}^{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Metabolic intrinsic clearance of M forming MG in liver</td>
<td>7.43 (16.5)</td>
<td>3.73 (28.3)</td>
</tr>
<tr>
<td>( f_{\text{met}}^{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Metabolic intrinsic clearance of M forming other metabolites in liver</td>
<td>0.35 (12.0)</td>
<td>0.51 (21.4)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net biliary clearance of M</td>
<td>0.06 (12.7)</td>
<td>0.13 (18.8)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net renal clearance of M</td>
<td>0.91 (54.5)</td>
<td>1.27 (57.8)</td>
</tr>
<tr>
<td>( f_{\text{QGut}} ) (ml( \cdot )min(^{-1}))</td>
<td>Renal clearance for M after correcting for ( f_{\text{b}}^{\text{M}} ) (Table 2)</td>
<td>1.39</td>
<td>1.94</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Efflux clearance for MG in the intestine</td>
<td>4.09 (24.1)</td>
<td>2.97 (47.7)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Intestinal intrinsic secretion for MG</td>
<td>0.22 (21.8)</td>
<td>0.35 (11.4)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Influx clearance for MG in the liver (assigned)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Efflux clearance for MG in the liver</td>
<td>0.04 (62.1)</td>
<td>0.2 (56.8)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Biliary clearance of MG</td>
<td>0.29 (38.3)</td>
<td>0.70 (43.5)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Net renal clearance of MG</td>
<td>2.40 (54.5)</td>
<td>0.40 (48.3)</td>
</tr>
<tr>
<td>( f_{\text{CL,M,L,Mint}} ) (ml( \cdot )min(^{-1}))</td>
<td>Renal clearance for MG after correcting for ( f_{\text{b}}^{\text{MG}} = 0.98 )</td>
<td>2.45</td>
<td>0.41</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike information criteria</td>
<td>317</td>
<td>308</td>
</tr>
</tbody>
</table>

### Table 6

Comparison of the goodness of fit among the three models

<table>
<thead>
<tr>
<th>Statistic Parameters</th>
<th>Two-Compartmental Model</th>
<th>TM-PBPK</th>
<th>SFM-PBPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weighted residual sum of squares</td>
<td>445</td>
<td>419</td>
<td>394</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Versus two-compartment model</td>
<td>— —</td>
<td>4.31*</td>
<td>7.83*</td>
</tr>
<tr>
<td>Versus TM-PBPK model</td>
<td>— —</td>
<td>— —</td>
<td>23.0*</td>
</tr>
</tbody>
</table>

*not applicable;
*Calculated F score > critical F value of 4.0, suggesting the order of goodness of fit: two-compartment model < TM-PBPK < SFM-PBPK.
TABLE 7
Additional parameters obtained from estimates in Table 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>TM-PBPK</th>
<th>SFM-PBPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{M}^{CL_{m,1}}$</td>
<td>Ratio of effective uptake/efflux clearance of $M$ in intestine, or intestine to blood partitioning of unbound morphine</td>
<td>0.137</td>
<td>0.531</td>
</tr>
<tr>
<td>$f_{M}^{CL_{m,1}}$</td>
<td>Ratio of effective uptake/efflux clearance of $M$ in liver, or liver to blood partitioning ratio of unbound morphine</td>
<td>1.42</td>
<td>2.46</td>
</tr>
<tr>
<td>$F_1$</td>
<td>Intestinal availability of $M$</td>
<td>0.654</td>
<td>0.457</td>
</tr>
<tr>
<td>$F_L$</td>
<td>Hepatic availability of $M$</td>
<td>0.574</td>
<td>0.574</td>
</tr>
<tr>
<td>$F_{sys} = F_{abs} \cdot F_1 \cdot F_L$</td>
<td>Systemic bioavailability:</td>
<td>0.334</td>
<td>0.242</td>
</tr>
<tr>
<td>$F_{(m)1}$</td>
<td>Intestinal availability of $MG$</td>
<td>0.95</td>
<td>0.89</td>
</tr>
<tr>
<td>$F_{(m)L}$</td>
<td>Hepatic availability of $MG$</td>
<td>0.12</td>
<td>0.22</td>
</tr>
<tr>
<td>$h_m$</td>
<td>Fraction of hepatic clearance of $M$ forming $MG$</td>
<td>0.498</td>
<td>0.854</td>
</tr>
<tr>
<td>$g_m = (1 - f_e)h_m$</td>
<td>Fraction of total body clearance of $M$ forming $MG$ (note that $f_e = 0.334$ from Table 3)</td>
<td>0.631</td>
<td>0.569</td>
</tr>
<tr>
<td>$\frac{v_I}{v_I + v_L}$</td>
<td>Fractional contribution of intestine to intestinal-liver removal</td>
<td>0.460</td>
<td>0.993</td>
</tr>
<tr>
<td>$\frac{v_L}{v_I + v_L}$</td>
<td>Fractional contribution of liver to intestinal-liver removal</td>
<td>0.570</td>
<td>0.171</td>
</tr>
</tbody>
</table>

*Based on definition of Pang and Kwan (1983).
*Calculated based on equations from Pang and Chow (2012): $\frac{v_I}{v_I + v_L} = f_{Q}\frac{(1 - F_1)}{f_{Q}(1 - F_1) + f_{Q}(1 - F_1) + F_{abs}}$.
*Calculated based on equations from Pang and Chow (2012): $\frac{v_L}{v_I + v_L} = f_{Q}\frac{1}{f_{Q}(1 - F_1) + F_{abs}}$.
*From Equations from Pang and Sun (2010).
*From equations from Pang and Sun (2010).
is the first of its kind to provide detailed information on metabolite kinetics.

Appendix A: Equations for Compartmental Modeling

Rate of change of M in gut lumen for intraduodenal dosing

$$\frac{dC_1}{dt} = -(k_{10} + k_{12})C_1V_1 + k_{A1}C_1 + k_{12}C_2V_2$$  \hspace{1cm} \text{for intraduodenal dosing}  \hspace{1cm} (2)$$

Rate of change of M in the peripheral compartment

$$\frac{dC_2}{dt} = -k_{21}C_1V_1 + k_{12}C_2$$  \hspace{1cm} (3)

Rate of change of MG or formed metabolite, denoted as \{mi\}

$$\frac{dC[mi]}{dt} = k_mC_1V_1 - k[mi]C[mi]V[mi]$$  \hspace{1cm} \text{for intravenous dosing}  \hspace{1cm} (2A)$$

Rates of biliary excretion of M and MG

$$\frac{dA_{bile}}{dt} = k_{bile}C_1V_1$$  \hspace{1cm} (5)

Rates of renal excretion of M and MG

$$\frac{dA_{renal}}{dt} = k_{renal}C_1V_1$$  \hspace{1cm} (7)

Appendix B: Equations for PBPK Modeling

Several assumptions were made: deglucuronidation of M was absent and reabsorption of MG was absent (Doherty and Pang, 2000). Once formed in the intestine or tissue, MG is effluxed out apically by Mrp2 or basolaterally by Mrp3 (van de Wetering et al., 2007) with efflux clearances CL_{MG}^{int} and CL_{MG}^{tissue,1}, respectively, for the intestine and liver. MG permeates through the liver basolateral membrane with rate of 1 ml/min^{-1} (Doherty et al., 2006) but not through the intestine membrane for secretion (Doherty and Pang, 2000).

Rate of change of M and MG in the blood compartment

$$V_B\frac{dM_B}{dt} = Q_{RP}M_{RP}K_{RP} + Q_{PP}M_{PP}K_{PP} + Q_{AD}M_{AD}K_{AD} + (Q_I + Q_{HA})M_{LB} - (Q_I + Q_{HA})M_B - f_{bM}M_BCL_R^{int}$$  \hspace{1cm} (9)

Rate of change of M in rapidly perfused tissue

$$V_{RP}\frac{dM_{RP}}{dt} = Q_{RP}M_B - Q_{RP}M_{RP}K_{RP}$$  \hspace{1cm} (11)

Rate of change of M in poorly perfused tissue

$$V_{PP}\frac{dM_{PP}}{dt} = Q_{PP}M_B - Q_{PP}M_{PP}K_{PP}$$  \hspace{1cm} (12)

Rate of change of M in adipose tissue

$$V_{AD}\frac{dM_{AD}}{dt} = Q_{AD}M_B - Q_{AD}M_{AD}K_{AD}$$  \hspace{1cm} (13)

For the Intestine and Liver according to the TM

Rates of change of M and MG in the intestine, I

$$\frac{dM_I}{dt} = f_A^{I}M_{IB}M_{IB} + f_A^{M}M_{I}(CL_{MG}^{int,mot,1} + CL_{MG}^{int,sec,1} + CL_{MG}^{mot,1}) + k_{IA}M_I$$  \hspace{1cm} (14)

Rates of change of M and MG in intestinal blood, IB

$$\frac{dM_{IB}}{dt} = Q_I(M_B - M_{IB}) + f_B^{M}M_{IB}(CL_{MG}^{I}M_{IB}^{mot,1} + CL_{MG}^{I}M_{IB}^{mot,1})$$  \hspace{1cm} (16)

$$\frac{dM_{IB}}{dt} = Q_I(M_B - M_{IB}) + f_B^{M}M_{IB}(CL_{MG}^{I}M_{IB}^{mot,1} + CL_{MG}^{I}M_{IB}^{mot,1})$$  \hspace{1cm} (17)
Rates of change of M and MG in the enterocyte, en

\[
\frac{dM_{en}}{dt} = f_B M_{ent} + f_M M_{inL} - f_M M_{enL} + f_l M_{enL} + \frac{dM_{en}}{dt} + \frac{dM_{mg}}{dt} \tag{18}
\]

For the Intestine and Liver according to the SFM

Rates of change of M and MG in the enterocyte, en

\[
\frac{dM_{en}}{dt} = f_B M_{ent} + f_M M_{inL} - f_M M_{enL} + f_l M_{enL} + \frac{dM_{en}}{dt} + \frac{dM_{mg}}{dt} \tag{22}
\]

Rates of change of M and MG in the enterocyte blood, enB

\[
\frac{dM_{enB}}{dt} = f_Q(m_B - M_{enB}) + f_M M_{enB} + f_B M_{entB} \tag{24}
\]

Rates of change of M and MG in serosa, s

\[
\frac{dM_s}{dt} = f_B M_{is} + f_M M_{isL} - f_M M_{es} + f_l M_{esL} \tag{26}
\]

Rates of change of M and MG in serosal blood, sB

\[
\frac{dM_{sB}}{dt} = (1 - f_Q) M_{iB} + f_M M_{iB} + f_B M_{isB} \tag{27}
\]

Rates of change of M and MG in liver blood, LB

\[
V_L \frac{dM_{LB}}{dt} = Q_H A M_B + f_Q Q_{MB} + (1 - f_Q) Q_{LB} M_B \tag{28}
\]

References


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Authorship Contributions

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Performed data analysis: Yang, Fan, Chen, Liu, Sun, Pang.

Wrote or contributed to the writing of the manuscript: Yang, Fan, Chen, Pang.


