Elucidation of Metabolic and Disposition Pathways for Maribavir in Nonhuman Primates through Mass Balance and Semi-Physiologically Based Modeling Approaches ^S

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ABSTRACT

Maribavir is in phase 3 clinical development for treatment of cytomegalovirus infection/disease in transplant recipients. Previous research conducted using only intact cynomolgus monkeys indicated biliary secretion as the primary elimination pathway for maribavir and that maribavir undergoes enterohepatic recirculation (EHR). To clarify the exact mechanisms of maribavir's EHR behavior, we studied its clearance pathways using intravenously administered ¹⁴C-labeled maribavir in intact and bile duct-cannulated (BDC) monkeys and constructed a semi-physiologically based pharmacokinetic (PBPK) model. Total radioactivity metabolite profiles in plasma and excreta were quantitatively determined along with plasma maribavir concentrations. Intact animals showed significantly lower clearance and longer half-lives in both total radioactivity and parent concentration in plasma than BDC monkeys. The primary in vitro and in vivo metabolic pathway for maribavir in monkey is direct glucuronidation; *N*-dealkylation and renal clearance are minor pathways. In BDC monkeys, 73% of dose was recovered as maribavir glucuronides in bile, and 3% of dose was recovered as parent in bile and feces; in intact animals' feces, 58% of dose was recovered as parent, and no glucuronides were detected. Therefore, EHR of maribavir occurs through biliary

secretion of maribavir glucuronides, and this is followed by hydrolysis of glucuronides in the gut lumen and subsequent reabsorption of parent. A semi-PBPK model constructed from physiologic, in vitro, and in vivo BDC monkey data is capable of projecting maribavir's pharmacokinetic and EHR behavior in intact animals after intravenous or oral dosing and could be applied to modeling other xenobiotics that are subject to similar EHR processes.

SIGNIFICANCE STATEMENT

Through both mass balance and semi-physiologically based pharmacokinetic (semi-PBPK) modeling approaches, this study mechanistically and quantitatively elucidates maribavir's enterohepatic recirculation (EHR) behavior in monkeys, which occurs via extensive direct glucuronidation, biliary secretion of these glucuronides, luminal hydrolysis of glucuronides to parent, and subsequent reabsorption of the parent. The study also identifies important drug- and animal-specific parameters that determine the EHR kinetics, and the semi-PBPK model is readily applicable to other drugs that undergo similar metabolic and recirculation mechanisms.

Introduction

Cytomegalovirus (CMV) infection is a serious complication that frequently occurs in recipients of hematopoietic cell or solid organ transplantations (Teira et al., 2016; Hakimi et al., 2017). Treatment with the currently available antiviral therapies has limitations, such as drug toxicities and lack of efficacy against drug-resistant strains of CMV (Venton et al., 2014; Kotton et al., 2018). Maribavir (5,6-dichloro-2-(isopropylamino)-1, β -L-ribofuranosyl-1-H-benzimidazole; structure shown in Fig. 1) is a potent and

selective orally bioavailable benzimidazole riboside and is active against human CMV (Biron et al., 2002; Williams et al., 2003) by blocking nuclear egress of viral capsids through protein kinase UL97 inhibition (Krosky et al., 2003; Hamirally et al., 2009); this mechanism of action stands in contrast to that of DNA polymerase inhibitors (ganciclovir, valganciclovir, cidofovir, and foscarnet) and terminase inhibitor (letermovir) approved for management of CMV (Marty et al., 2017; Kotton et al., 2018). In two phase 2 studies, the majority of solid organ transplant and hematopoietic stem cell transplant recipients with CMV infection achieved viremia clearance after maribavir treatment across all doses studied [400, 800, and 1200 mg twice daily (b.i.d.)]; maribavir exhibited comparable efficacy to valganciclovir, and recipients of maribavir experienced low incidences of neutropenia and renal toxicity (Maertens et al., 2019; Papanicolaou et al., 2019). The ongoing clinical development program for maribavir for the treatment of transplant recipients with CMV includes two phase 3 trials (NCT02927067, NCT02931539) (National Library of Medicine, 2020a,b).

The pharmacokinetics (PK), metabolism, and disposition of maribavir in nonclinical species have been previously reported (Koszalka et al., 2002).

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ABBREVIATIONS: AUC, area under the curve; BDC, bile duct-cannulated; b.i.d., twice daily; BLQ, below the limit of quantitation; CMV, cytomegalovirus; EHR, enterohepatic recirculation; f_m , fraction metabolized; f_m (Gluc), fraction metabolized by direct glucuronidation; GI, gastrointestinal; GUS, β -glucuronidase; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LSC, liquid scintillation counter; PBPK, physiologically based pharmacokinetic(s); %PE, percentage predictive error; P_{eff} , effective permeability; PK, pharmacokinetic(s); p.o., orally; SITT, small intestine transit time; UGT, UDP-glucuronosyltransferase.

Fig. 1. Structure of maribavir (also known as TAK-620, SHP620, VP41263, and 1263W94). The position of the ^{14}C atom is marked with an asterisk.

After intravenous or oral administration of ^{14}C -labeled maribavir to both intact rat and monkey, a large proportion of radioactivity ($\geq 89\%$ in rat and up to 57% in monkey) was recovered as unchanged parent in feces, suggesting that biliary excretion may be the predominant route of elimination in these species. Renal clearance was thought to be a minor elimination pathway for maribavir, as indicated by the relatively low percentage dose recovered in urine, at $<6\%$ total dose in rat and $<18\%$ total dose in monkey; a portion of the radioactivity in urine was attributed to the *N*-dealkylated metabolite VP44469 [4%–7% total dose in both rat and monkey after intravenous or oral (p.o.) dosing] (Koszalka et al., 2002). VP44469 was also detected in feces of monkeys (11% intravenous dose and 15.5% p.o. dose). Additionally, in these monkeys, a prolonged elimination phase was observed, suggesting that maribavir may undergo enterohepatic recirculation (EHR) before excretion. Biliary clearance was thus deemed the major clearance pathway and oxidative metabolism to VP44469 as the primary metabolic pathway, as indicated by parent and metabolite profiles in plasma, urine, and feces of intact animals (Koszalka et al., 2002).

Characterization of clearance pathways of xenobiotics is commonly evaluated using bile duct–cannulated (BDC) animals most often in rats but also larger animal species (Kimoto et al., 2017). The use of only intact animals in previous research (Koszalka et al., 2002) that pointed to biliary secretion being the primary elimination pathway for maribavir confounds determination of the mechanisms of its EHR. A lack of understanding on such mechanisms presents inherent uncertainty pertaining to evaluation of drug–drug interaction risks and factors driving interindividual variability in maribavir PK. Here we conducted additional definitive studies in monkeys, which were selected because of their relative closeness in physiology to humans, particularly in organs, such as liver and the gastrointestinal tract, and present quantitative evidence on the clearance pathways of maribavir in both intact and BDC monkeys as well as the mechanism of EHR in intact monkeys. We then constructed a semi–physiologically based pharmacokinetic (semi-PBPK) model and demonstrated that the PK of maribavir in intact monkeys could be projected with physiologically based modeling and simulations and also identified important parameters that drive the extent and kinetics of EHR of maribavir with implications in both nonhuman primates and humans.

Materials and Methods

Materials

Maribavir was provided by Carbogen AMCIS AG (Bubendorf, Switzerland). ^{14}C -maribavir (2.43 $\mu\text{Ci}/\text{mg}$) and d_7 -maribavir were provided by Almac Sciences (Craigavon, Northern Ireland, United Kingdom). The chemical purity and radioactive purity of ^{14}C -maribavir (lot SJJ-0004E-010-01) were $>98\%$. Cryopreserved mixed-sex pooled human hepatocytes (20 donors) and cynomolgus

monkey hepatocytes (6 donors) were purchased from BioreclamationIVT (Baltimore, MD). All buffers and chemicals used in *in vitro* studies were obtained from Sigma-Aldrich (St Louis, MO) or EMD Chemicals (Gibbstown, NJ).

Animal Preparation and Dosing

Animals. All animal housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by US National Institutes of Health and were approved by the Institution's Animal Care and Use Committee or local equivalent. Male cynomolgus monkeys were from the Covance (Princeton, NJ) stock colony. Animals were acclimated to study conditions for 5 weeks prior to dose administration. At dosing, animals weighed between 3.7 and 4.2 kg and were 3–4 years of age. Animals were assigned to either group 1 (intact, $n = 3$) or group 2 (BDC, $n = 3$). BDC surgery was done under sterile conditions using appropriate sedation and inhalation anesthetic. The bile duct was cannulated to allow collection of bile, and a second cannula was placed in the duodenum for the infusion of bile salts replacement solution or other fluids (Kimoto et al., 2017). Further details on animal husbandry and surgical procedures are provided in the Supplemental Material.

Dosing. ^{14}C -Maribavir dosing solution was prepared on the day of dosing. Animals were dosed with 13 mg/kg ^{14}C -maribavir (corresponding to a mean 31.5 $\mu\text{Ci}/\text{kg}$; prepared in 7% ethanol and 15% propylene glycol in sterile saline vehicle) via intravenous infusion followed by a 2-ml flush of saline. Details on the preparation and administration of ^{14}C -maribavir are provided in the Supplemental Material.

Pharmacokinetics and Excretion Balance

Sample Collection. Blood was collected from both groups (intact and BDC) at predose and at 5, 15, and 30 minutes, and this was followed by 2, 6, 24, 48, 72, 96, 120, 144, and 168 hours postdose. At each time point, blood samples were collected from each animal for radioanalysis and metabolite profiling (1 ml) and plasma extraction (1.5 ml).

Blood samples were processed using standard techniques (Supplemental Material) and stored at -70°C until analysis. Urine and feces from both groups and bile (group 2) were collected at 0–8 and 8–24 hours postdose and at 24-hour intervals through to at least 168 hours postdose. Nonbiologic samples (cage rinse, cage debris, cage wash, cage wipe, jacket extract, and urine wipe; bile duct cannulae for group 2) were also collected. Further details can be found in the Supplemental Material.

Radioactivity Measurement. Radioactivity in samples was measured using Model 2900TR and 2910TR liquid scintillation counters (LSCs; Packard Instrument Company, Downers Grove, IL) with Ultima Gold XR scintillation cocktail (PerkinElmer, Waltham, MA) for at least 5 minutes or 100,000 counts. Blood and fecal samples were further processed before analysis (see Supplemental Material). Other samples (including bile, urine, and plasma) were analyzed directly. To obtain disintegration-per-minute data, scintillation counting data (in cpm) were automatically corrected for counting efficiency using the external standardization technique, and an instrument-stored quench curve was generated from a series of sealed quenched standards. The representative lower limit of quantitation for radioactivity in blood and plasma was 40-ng equivalent/g for 72-hour samples in group 1, 24-hour samples in group 2, and 78-ng equivalent/g for all other blood and plasma samples.

Metabolite Profiling

Radioactivity Extraction Recovery. Extraction recoveries for each excreta were determined as described in the Supplemental Material.

Plasma. Plasma samples were pooled by group and at each time point. Radioactivity in each pooled sample was determined by LSC. Reconstituted samples (see Supplemental Material) were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) with eluent fractions collected at 10-second intervals into 96-well plates containing solid scintillant. Radioactivity in each well was determined using TopCount analysis for the generation of radioactive profiles.

Urine. Predose to 144-hour samples from group 1 and predose to 48-hour samples from group 2 were analyzed using liquid chromatography–mass spectrometry (LC-MS) with eluent fractions collected at 10-second intervals into 96-well plates containing solid scintillant. Radioactivity in each well was determined

using TopCount analysis, and radiochemical profiles were generated based on radioactivity counts. Due to low levels of radioactivity, group 1 samples from 144 hours onward and group 2 samples from 48 hours onward were analyzed by LC-MS/MS only.

Bile. Bile samples from group 2 were pooled by collection interval. Radioactivity in each pooled sample was determined by LSC. Samples were analyzed by LC-MS/MS, and radioactivity was determined as described above.

Feces. Feces from group 1 were pooled to generate one 0–120-hour pooled sample. Samples from group 2 were pooled to generate one 0–72-hour pool. Radioactivity in each pooled sample was determined by LSC. Reconstituted samples (see Supplemental Material) were analyzed by LC-MS/MS with eluent fractions collected at 10-second intervals into 96-well plates containing solid scintillant. The radioactivity in each well was determined through TopCount analysis, and radioactive profiles were generated based on radioactivity counts.

LC/MS-MS Instrumentation and Conditions. Processed tissue matrices samples were injected with a Shimadzu Nexera SIL-30ACMP autoinjector (10°C) equipped with a Prominence CBM-20A controller and Nexera LC-30AD pumps (Shimadzu Scientific Instruments, Columbia, MD) that was coupled to a Phenomenex 3 × 4 mm C18 guard column (Phenomenex, Torrance, CA), a Waters Atlantis T3 HPLC column (4.6 × 250 mm, 5 μm; Waters Corporation, Milford, MA), and an LEAP Technologies PAL HTC-xt fraction collector (LEAP Technologies Inc., Morrisville, NC). Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. Tandem mass spectrometry (MS/MS) was performed in a Thermo Fisher Scientific Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with a positive/negative heated electrospray interface. Detailed LC-MS/MS conditions are listed in Supplemental Material.

Data Analysis for Mass-Balance Study in Monkey

Pharmacokinetic and Mass-Balance Analyses. Radioanalysis data tables were generated by Debra, version 6.1.1.87 (LabLogic Systems Ltd., Sheffield, UK). PK parameters for both total radioactivity and plasma concentrations of parent were calculated using Phoenix WinNonlin, version 6.4 or higher (Certara USA, Inc., Princeton, NJ) with a noncompartmental approach.

Metabolite Identification. To describe and quantify peaks, a background of 3 cpm was applied to all chromatograms, and the net radioactivity in each peak was expressed as a percentage of total radioactivity in the chromatogram or sample. Metabolite profiles in plasma were reported as a percentage total sample radioactivity and as concentration (ng equivalents/g). For excreta, the percentage of the administered dose excreted as the component represented by the peak was calculated using the following equation: % of dose = % of radioactivity in peak × % of dose in sample. The percentage dose and concentration of the peak were corrected for extraction and reconstitution recoveries as applicable. M numbers were assigned to peaks as M1 through M6 to match previously reported metabolites along with additional metabolites found in this study assigned up to M17.

Pharmacokinetics of Unlabeled Maribavir in Intact Monkeys after a Single Intravenous or Oral Administration

Male cynomolgus monkeys of Chinese origin aged 3–4 years ($n = 3$) were used. On day 1 each animal received a single intravenous dose of maribavir at 5 mg/kg in a dose volume of 1 ml/kg in saline containing 10% ethanol and 40% propylene glycol 400. Blood was collected for plasma at predose and at 0.083, 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 36, 48, 72, and 96 hours postdose. On day 15, the same animals received a single oral gavage dose of maribavir at 10 mg/kg in a dose volume of 5 ml/kg with the same vehicle as intravenous dosing. The feeding tube was flushed with 2–3 ml of water after gavage dosing. Blood was collected for plasma once predose and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 24, 36, 48, 72, and 96 hours postdose. Monkey plasma samples added with the internal standard (d_7 -maribavir) were extracted using protein precipitation with organic solvent. Plasma samples were analyzed by high-performance liquid chromatography equipped with an AB SCIEX API 4000 triple quadrupole mass spectrometer using an electrospray ionization source. Negative ions were monitored in the multiple reaction monitoring mode. Quantitation was determined using a weighted linear regression analysis ($1/\text{concentration}^2$) of peak area ratios of the maribavir and internal standard. Additional details are in the Supplemental Material. Noncompartmental PK analysis was performed for both the intravenous and p.o. arms with Phoenix WinNonlin version 6.4 or higher. The same noncompartmental analysis was also used to calculate PK parameters for previously published studies with maribavir.

In Vitro Metabolism of ^{14}C -maribavir in Hepatocytes

The in vitro metabolism of maribavir was evaluated in cryopreserved hepatocytes isolated from cynomolgus monkeys or humans. ^{14}C -Maribavir (10 μM) was incubated in 1 ml at 10^6 cells/ml for 4 hours, and metabolite profiles were obtained by analyzing hepatocyte extracts by LC/UV/MS and LC/UV/radioactivity flow detector. Further details are described in the Supplemental Material.

Permeability of Maribavir across Caco-2 Cell Monolayer

Caco-2 cell monolayers were grown to confluence on collagen-coated, micro-porous, polycarbonate membranes in 12-well Transwell plates (Corning Life Sciences, Tewksbury, MA). Transepithelial electrical resistance was measured with standard procedures before and after the study (Benson et al., 2013); the average transepithelial electrical resistance was 553 Ω·cm². Compounds tested were maribavir as well as Lucifer Yellow, atenolol, and propranolol. Cells were dosed on the apical or basolateral side and incubated at 37°C. At 1 and 2 hours, a 200-μl aliquot was taken from the receiver chamber and replaced with fresh assay buffer. Concentrations of test article were determined by LC-MS in ES+ mode. Further details are described in the Supplemental Material.

Semi-PBPK Model for Disposition of Maribavir in Monkeys

Based on findings from the mass-balance study in BDC and intact monkeys, a semi-PBPK model was developed to describe the kinetics of maribavir in plasma and that of maribavir and its glucuronides in the gastrointestinal (GI) tract. The model construction, parameter estimation, simulations, and sensitivity analyses were conducted with SimBiology version 5.7 hosted in MATLAB R2017b with Optimization Toolbox version 8.0 (The Mathworks Inc., Natick, MA).

Model Construction. The semi-PBPK model comprised a compartmental absorption and transit module for the GI tract (Yu and Amidon, 1999) and three systemic compartments (peripheral, central, and liver) (Fig. 2; Supplemental Fig. 1; Supplemental Tables 1 and 2). The GI model consists of 13 total luminal compartments: stomach (one compartment), duodenum (one), jejunum (two), ileum (four), and colon (five). Within each luminal segment, maribavir is absorbed with first-order kinetics, with the rate (k_a) determined by effective permeability (P_{eff}) specific to each segment (see Supplemental Methods and Supplemental Table 3). Human jejunal P_{eff} of maribavir was estimated from its measured Caco-2 apparent permeability, which was calibrated by assay controls (atenolol and propranolol) and log-linear regression of $P_{\text{eff}}\text{-}P_{\text{app}}$ (apparent permeability) data (see Supplemental Fig. 2; Supplemental Methods; Supplemental Table 4). Monkey jejunal P_{eff} was assumed to be the same as human. The observed plasma concentrations were assumed to resemble that of the central compartment. The liver compartment was necessary to model the first-pass metabolism as well as the metabolic and biliary elimination of maribavir as glucuronides or others. Luminal conversion of maribavir glucuronides to maribavir was assumed to occur in the distal small intestine and throughout the colon, and it was also assumed that the entirety of regenerated maribavir is available for reabsorption. All system parameters, such as intestinal transit times, intestinal radii, and hepatic blood flow, were obtained from literature; drug-specific parameter values were determined in vitro studies, measured from excreta contents of the BDC group, or estimated from the plasma concentration time course of the BDC group (Supplemental Table 5; Table 1). Further details are described in the Supplemental Material.

Parameter Estimation. Four systemic PK parameters [drug clearance from the liver compartment (Drug_Liver_CL), central-to-peripheral transfer clearance (Drug_Q12), and volumes of the central and peripheral compartments (Drug_Vc_Ref and Drug_Vp_Ref)] were estimated by fitting the BDC group plasma concentrations of maribavir to the central compartment concentration using the constrained nonlinear least-squares method. The observed plasma concentrations were pooled at each time point (because of BDC animal 2 having fewer available plasma concentrations), and an exponential error model was chosen. Termination tolerance on the estimated coefficients, objective function value, and first-order optimality were all set at 10^{-12} , and maximum iterations were set at 5000. The performance of fitting was evaluated and confirmed with %CV of the estimated values (Table 1) and the observed data versus predictions and residuals versus predictions plots (Supplemental Fig. 3, A and B).

Model Qualification and Simulations. By varying the doses and routes of administration (intravenous bolus or oral), plasma concentration versus time profiles of maribavir in intact animals were simulated using the semi-PBPK model for 13 mg/kg i.v. dosing in mass-balance study; for 5 mg/kg i.v. and 10 mg/kg p.o. dosing in the unlabeled PK study; and for 20, 60, and

Fig. 2. Simplified diagram for the semi-PBPK model of maribavir disposition in cynomolgus monkeys. Numbers in the graph denote physiologic and biologic processes: (1) transit of substance from stomach to duodenum; (2) transit within the small intestine; (3) transit within the colon and to feces; (4) absorption from the small intestine; (5) conversion of glucuronides to parent, which occurs in the last two compartments of the ileal lumen and throughout the colon; (6) absorption from the colon; (7) biliary excretion of parent; (8) glucuronidation of parent and secretion to duodenal lumen; (9) excretion of glucuronides in urine; (10) transport between the liver and central compartment; (11) transport between the central and peripheral compartments; (12) renal excretion of parent; and (13) all other pathways for parent. The two segments of the jejunum, four segments of the ileum, and five of the colon were not separately depicted in this diagram; the detailed diagram in MATLAB SimBiology is shown in Supplemental Fig. 1. MBV, maribavir (parent); MBV-Gluc, maribavir glucuronides; Met, other metabolites of maribavir.

180 mg/kg/day reported in a historical toxicokinetic study (Koszalka et al., 2002). The SUNDIALS solver was used, and absolute tolerance was set at 1.0^{-9} ; dimension analysis was enabled. Observed data were overlaid to and graphically compared with simulated results. Quantitative comparisons included area under the curve (AUC) (intravenous and p.o.), C_{max} , time to peak concentration (T_{max}), and bioavailability (p.o. only) as well as the median percentage predictive error (%PE, defined by eq. 1):

$$\%PE = \frac{C_{Pred} - \bar{C}_{Obs}}{\bar{C}_{Obs}} \times 100\%$$

in which $\bar{C}_{Obs}$ is the mean observed plasma concentration at each time point, and C_{Pred} is the simulated concentration at corresponding time points. A median %PE between -50% and 100% , which corresponds to the predicted value being within the 2-fold range of the mean observed value, generally indicates a PBPK model with good predictability (Khalil and Laer, 2014; Zhou et al., 2016; Matsumoto et al., 2019).

Sensitivity Analysis. Sensitivity analyses of the plasma PK time profile of maribavir after a single 10-mg/kg p.o. administration with respect to parameters in Table 1 were conducted within SimBiology by varying each designated parameter within a range around the final model value. Four drug- and system-specific parameters [i.e., P_{eff} in the jejunum, clearance from the liver compartment (Drug_Liver_CL), fraction metabolized (f_m) by the direct glucuronidation pathway ($f_m(\text{Gluc})$), and transit rate within the colonic lumen (k_{T_Colon})] were found to confer the most impact on the exposure of maribavir.

Results

Metabolic Pathway of ^{14}C -Maribavir after a Single Intravenous Bolus Injection to Cynomolgus Monkeys

After a single 13-mg/kg i.v. dose of ^{14}C -maribavir to male intact monkeys (group 1), radioactivity in plasma declined after the first time point (5 minutes) and was below the limit of quantitation (BLQ) after 72 hours (Fig. 3A; Supplemental Fig. 4). Mean clearance and terminal half-life ($t_{1/2}$) for radioactivity in plasma were 1700 g/h and 19.7 hours,

respectively (Table 2). In BDC monkeys (group 2), after a single 13-mg/kg i.v. dose of ^{14}C -maribavir, radioactivity in plasma declined more rapidly than in group 1 and was BLQ by 24 hours postdose (Fig. 3A; Supplemental Fig. 4). In group 2, the clearance of radioactivity in plasma at 3010 g/h was 77% higher than in group 1; mean $t_{1/2}$ (2.71 hours) was also much shorter than in group 1 (Table 2).

The major route of elimination of radioactivity in intact monkeys (group 1) was via feces, with a mean of 75.2% excreted over 336 hours (Fig. 4A). The mean percentage of radioactivity recovered in urine was 14.1% over 336 hours, with the majority of the radioactivity being excreted by 96 hours. The major route of elimination of radioactivity in BDC monkeys (group 2) was via bile, with a mean of 84.0% excreted over 168 hours postdose (Fig. 4B). The mean percentages of the administered radioactivity recovered in urine and feces from BDC monkeys were 5.16% and 2.36%, respectively, over 168 hours. The majority of the radioactivity was excreted by 8 hours postdose; radioactivity levels in bile were BLQ by 72 hours. The mean combined radioactivity in all nonbiologic samples was less than 5% of dose for both groups. The mean overall recovery of the dose administered was 95.3% and 97.0% in groups 1 and 2, respectively.

Pharmacokinetics of ^{14}C -Maribavir in Plasma after Intravenous Bolus Injection to Monkeys

The PK profiles of maribavir in plasma after a single intravenous bolus administration in monkeys are depicted in Fig. 3B (individually plotted in Supplemental Fig. 4), and the PK properties are listed in Table 3. The mean clearance in intact animals (2.99 l/h) was about half of that in BDC animals (5.72 l/h). Intact animals also displayed a much more pronounced terminal phase (mean $t_{1/2}$ of 12.5 hours) than their BDC counterparts ($t_{1/2}$ 2.5 hours). These results were consistent with the aforementioned observations on total radioactivity (Fig. 3A; Supplemental Fig. 4; Table 2).

TABLE 1

Key system parameters and drug-specific parameters used in construction of the customized semi-physiologically based pharmacokinetic model for maribavir in cynomolgus monkeys

For a full list of parameters, refer to Supplemental Table 5. "Drug" refers to maribavir.

Parameter in SimBiology	Description	Value	Notes
Key System Parameters			
k_T_Stomach	Rate of transit from stomach to duodenum	2.0 h ⁻¹	From fasted state, gastric emptying time of 30 min
SITT	Small intestine transit time	2.7 h	Ikegami et al., 2003
k_T_Colon	Colon transit rate	0.042 h ⁻¹	Peters et al., 2012
Key Drug-Specific Parameters from In Vitro Data or In Vivo BDC Group Data			
Drug_Peff	Effective permeability in jejunum	1.3 × 10 ⁻⁴ cm/s	Calculated from Caco-2 cell data; see Supplemental Methods
Drug_ka_Jejunum	First-order absorption rate in jejunum	2.3 h ⁻¹	Calculations in Supplemental Methods
Drug_ka_Colon	First-order absorption rate in colon	0.056 h ⁻¹	Calculations in Supplemental Methods
Drug_fm_gluc	Fraction metabolized by the direct glucuronidation pathway	0.73 or 0.85	0.728 from in vivo data in BDC group (Table 5); 0.853 from in vitro hepatocyte data
Gluc_k_hydrolysis	Rate of hydrolysis of maribavir glucuronides	3.1 h ⁻¹	Calculations in Supplemental Methods
Drug_CL_Renal	Renal clearance of parent from central compartment	0.013 l/h	From BDC mean systemic CL × %dose in urine: 5.72 l/h × 0.224% (Tables 3 and 4)
Drug_CL_Biliary	Biliary clearance of parent from central compartment	0.072 l/h	From BDC systemic CL × %dose in bile: 5.72 l/h × 1.26% (Tables 3 and 4)
Drug-Specific Parameters from Fitting the Model to the Observed Plasma Concentration Time Course in BDC Animals			
Drug_Q12	Central-to-peripheral transfer CL	0.66 l/h	S.E. 0.0049
Drug_Liver_CL	Drug clearance from the liver compartment	16 l/h	S.E. 0.24
Drug_Vc_Ref	Volume of the central compartment	6.3 l	S.E. 0.085
Drug_Vp_Ref	Volume of peripheral compartment	2.8 l	S.E. 0.046

CL, clearance.

Identification and Quantitation of Metabolites of ¹⁴C-Maribavir in Monkey Plasma and Excreta

Eleven metabolites of maribavir (Table 4; structures shown in Supplemental Table 6; MS/MS spectra and structural assignment for each fragment ion detailed in Supplemental Figs. 5–17) were tentatively identified from the collected biologic matrices (reconstructed ion chromatograms and radiochromatograms detailed in Supplemental Figs. 18–27). A comprehensive illustration of metabolic pathways of maribavir in monkeys is depicted in Fig. 5. For plasma from both intact and BDC animals, parent was the predominant species associated with radioactivity in circulation at almost all time points, accounting for more than 80% of all radioactivity in plasma (Supplemental Table 7). Much lower concentrations of the *N*-dealkylated metabolite M4 [also known as VP44469 (Goldwater et al., 2008)], two glucuronides of M4 (M7 and M17) and two direct glucuronides of parent (M11 and M12) were also detected at some time points in plasma, but the combined percentages of these metabolites generally amounted to less than 15% of circulating radioactivity. At all time points, no individual metabolite accounted for more than 8% of circulating radioactivity in plasma.

A pronounced difference in the composition of metabolites in excreta was observed between intact and BDC animals. In the excreta from intact animals (group 1), the bulk of radioactivity (58%–60% of the administered dose) was recovered as the unchanged parent compound in feces. The dealkylation product M4 generated from cytochrome P450-mediated pathways (Koszalka et al., 2002; Goldwater et al., 2008) represented approximately 9% of the total dose in pooled feces; in pooled urine, M4 and its glucuronides in total also comprised up to 9% of administered dose (Table 4). Metabolites M11 and M12, direct glucuronides of maribavir,

were present in the urine from intact animals at a combined 3% total dose. Neither maribavir glucuronides nor M4 glucuronides were detected in the feces of intact animals. In contrast, in BDC animals, the majority (73%) of administered dose was recovered as M11 and M12 in bile. Biliary secretion of the parent compound itself only accounted for a small fraction (1.3%) of the dose; the amount of parent in pooled feces of BDC animals was also small (1.5% total dose). In the urine of BDC monkeys, 0.22% of the dose was identified as parent, and M4 and its glucuronides combined were 3.6% of administered dose; thus, both values were numerically lower than the respective 0.89% and >7.1% observed in intact animals (Table 4). M15, a dechlorinated and cysteine-conjugated metabolite, was unique to BDC animals and detected only in bile samples, but it cumulatively represented only 1.4% of the total dose. Two other metabolites were also detected at low amounts: metabolite M5, with oxidation on *N*-isopropyl, at <0.43% of the total dose, and M2, the deribosylated and glucuronidated metabolite, at < 2.5% of the total dose in both groups (Table 4).

In Vitro Metabolism of Maribavir in Cryopreserved Hepatocytes

After a 4-hour incubation with cynomolgus monkey hepatocytes, ¹⁴C-maribavir was almost completely metabolized. Major metabolites included M11 and M12 (direct glucuronides) as well as the multiple glucuronides to M4 (*N*-dealkylation). M11 and M12 combined and M4 glucuronides represented 85.3% and 10.4% of total radioactive peak areas, respectively. After a 4-hour incubation with human hepatocytes, parent, M4, M4-glucuronides, and one of the direct glucuronides to parent (M11 or M12) represented 47.2%, 31.1%, 10.2%, and 10% of total radioactive peak areas, respectively; M4 plus its glucuronides

Fig. 3. (A) Total radioactivity in plasma vs. time profile after a single intravenous bolus administration of 13 mg/kg ¹⁴C-maribavir to intact animals (group 1, circles) or BDC animals (group 2, squares) as determined by LSC. (B) Concentration of maribavir in plasma vs. time profile after a single intravenous bolus administration of 13 mg/kg ¹⁴C-maribavir to intact animals (group 1, circles) or BDC animals (group 2, squares) as determined by LC-MS/MS. Insets show a zoomed-in view of the time course from zero to 6 hours postdose. Missing samples are not plotted, and BLQ data are treated as zeros. *N* = 3 for both groups, error bars denote standard deviation, and *N* = 2 for group 2's 24-hour sample in panel (B).

and M11/M12 represented 78% and 19% of metabolism of maribavir in human hepatocytes. Other metabolites were present at trace amounts (each <1%) for both monkey and human hepatocyte incubations.

Permeability of Maribavir Across Cultured Caco-2 Cell Monolayer

The apparent permeability for maribavir in the apical-to-basolateral and basolateral-to-apical directions were 5.9×10^{-6} cm/s and 33.7×10^{-6} cm/s, respectively. The apical-to-basolateral apparent

TABLE 2		
Noncompartmental PK parameters for radioactivity in plasma collected from male cynomolgus monkeys after a single intravenous administration of ¹⁴ C-maribavir (both groups at 13 mg/kg). Group 1: intact monkeys (<i>n</i> = 3); group 2: BDC monkeys (<i>n</i> = 3)		
PK Parameter on Total Radioactivity in Plasma	Group 1 (Intact), Mean ± S.D.	Group 2 (BDC), Mean ± S.D.
AUC ₀₋₄ (ng maribavir equivalent-h/g)	28,000 ± 5770	17,100 ± 4050
AUC _{0-∞} (ng maribavir equivalent-h/g)	30,300 ± 5900	17,900 ± 3470
<i>t</i> _{1/2} (h)	19.7 ± 3.4	2.71 ± 1.4
CL (g/h)	1700 ± 347	3010 ± 528
<i>V</i> _{ss} (g)	27,700 ± 3910	6010 ± 1110

AUC₀₋₄, area under the conc.-time curve from time 0 to the last measurable time point; AUC_{0-∞}, area under the conc.-time curve from zero to infinity; CL, clearance; *V*_{ss}, volume of distribution at steady state.

Fig. 4. Mean cumulative recovery percentages in urine, feces, cage rinse, and bile (group 2 only) as well as in all matrices combined ("TOTAL") in (A) intact animals (group 1) and (B) bile duct-cannulated animals (group 2) after a single intravenous bolus administration of ^{14}C -maribavir. $N = 3$ for both groups, and error bars denote standard deviation.

permeability for Lucifer Yellow, atenolol, and propranolol were 0.26×10^{-6} cm/s, 0.36×10^{-6} cm/s, and 17.1×10^{-6} cm/s, respectively.

Semi-PBPK Model for EHR of Maribavir in Monkeys

Parameter Estimations. The final PK parameters derived from fitting the semi-PBPK model to the observed plasma concentration over time from BDC monkeys (group 2) are listed in Table 1 (see also Supplemental Results and Discussion). The fitted concentration and observed data over time are shown in Fig. 6A; observed versus predicted and residual versus prediction graphs are in Supplemental Fig. 3, A and B. The overall residual error of the fitting was 0.256.

TABLE 3

Noncompartmental PK parameters for conc. of maribavir in plasma from male cynomolgus monkeys after a single intravenous bolus administration of ^{14}C -maribavir [groups 1 ($n = 3$) and 2 ($n = 3$), 13 mg/kg].

PK Parameter on Maribavir Concentration in Plasma	Group 1 (Intact), Mean $\pm$ S.D.	Group 2 (BDC), Mean $\pm$ S.D.
AUC_{0-t} (h·mg/l)	17.4 ± 3.9	9.33 ± 1.7
$\text{AUC}_{0-\infty}$ (h·mg/l)	17.4 ± 3.9	9.36 ± 1.6
C_0 (mg/l)	9.96 ^a	10.4 ± 1.3
$t_{1/2}$ (h)	12.5 ± 1.1	2.49 ± 1.2
CL (l/h)	2.99 ± 0.73	5.72 ± 0.90
V_{ss} (l)	35.4 ± 6.5	7.09 ± 1.5

AUC_{0-t} , area under the conc.-time curve from time 0 to the last measurable time point; $\text{AUC}_{0-\infty}$, area under the conc.-time curve from zero to infinity; C_0 , initial conc.; CL, clearance; V_{ss} , volume of distribution at steady state.

^aFor C_0 , only two animals in group 1 were involved in calculating the mean because of one animal missing the 5-minute PK time point.

Simulations for Intravenous Bolus Dosing of Maribavir in Monkeys. Two $f_m(\text{Gluc})$ values were used in simulations: 0.853 from the in vitro hepatocyte data or 0.728 from the metabolite content in the pooled bile of BDC monkeys. Graphic results for simulated and observed plasma concentrations of maribavir in intact monkeys administered with ^{14}C -maribavir (13 mg/kg mass equivalent) or 5 mg/kg unlabeled maribavir are shown in Fig. 6B and derived PK properties in Table 5. At both doses, the simulations were able to predict the initial phase of rapid decline (0 to 4–6 hours postdose) as well as the prolonged terminal phase (after 4–6 hours postdose) in maribavir plasma concentrations. The observed intergroup difference in predictability of the time course could potentially be explained by the contribution of glucuronidation in the overall clearance: Simulation with $f_m(\text{Gluc}) = 0.853$ and 0.728 seemingly presented a better match for terminal phase and AUC for the 13-mg/kg i.v. group and the 5 mg/kg i.v. group, respectively. The median %PE for 13 mg/kg i.v. was 12% at $f_m(\text{Gluc}) = 0.853$ and -49% at $f_m(\text{Gluc}) = 0.728$; median %PE for 5 mg/kg i.v. was 81% at $f_m(\text{Gluc}) = 0.853$ and 30% at $f_m(\text{Gluc}) = 0.728$. The overall median %PE for all available PK data points after intravenous dosing in intact animals was 38% at $f_m(\text{Gluc}) = 0.853$ and 1% at $f_m(\text{Gluc}) = 0.728$. The $f_m(\text{Gluc})$ of 0.728 was eventually selected for all follow-up simulations because it is a direct in vivo measurement and also produced a slightly better overall %PE and predictive profile across the concentration range (Supplemental Fig. 28).

Simulations for Oral Dosing of Maribavir and Qualification of the Semi-PBPK Model in Monkeys. The semi-PBPK model with $f_m(\text{Gluc}) = 0.728$ was able to capture the plasma concentration profiles after a single 10-mg/kg p.o. dose to intact monkeys (Fig. 6C) during both the apparent absorption and terminal phases; median %PE was 38%. Derived PK parameters, such as AUC,

TABLE 4

Descriptions and cumulative percentages of dose recovered in each pooled biologic matrix for maribavir and its metabolites after a single intravenous bolus administration to intact (group 1) or BDC (group 2) cynomolgus monkeys.

Metabolite	Description	Percentage of Dose Administered to Group 1		Percentage of Dose Administered to Group 2		
		Urine (0–144 h)	Feces (0–168 h) ^a	Urine (0–48 h)	Feces (0–48 h)	Bile (0–24 h)
M15	Loss of chlorine + cysteine conjugate	ND	ND	ND	ND	1.42
M1	Glucuronide conjugate to M4	2.16	ND	1.19	ND	ND
M7	Glucuronide conjugate to M4	2.62	ND	1.14	ND	3.77
M16	Glucuronide conjugate to M4	0.297	ND	ND	ND	0.992
M17 ^b	Glucuronide conjugate to M4	0–1.85	ND	0–0.596	ND	0–1.83
M2 ^b	Loss of ribose + glucuronide	0–1.85	ND	0–0.596	0.0282	0–1.83
M10	Glucuronide conjugate to parent	0.402	ND	ND	ND	ND
M4	Loss of propyl	2.0	8.80–9.34	0.678	0.511	ND
M5	Oxidation on propyl	ND	0.416–0.433	0.0457	ND	ND
M11	Glucuronide conjugate to parent	1.2	ND	0.334	ND	34.2
M12	Glucuronide conjugate to parent	2.01	ND	0.551	ND	38.6
Maribavir	Parent	0.823	57.9–60.3	0.224	1.45	1.26

ND, not detected or below the limit of quantitation (1% of run and 10 cpm peak height).

^aThe lower dose percentage value was based on pooled fecal samples from all three animals from 0 to 120 hours. Only one animal generated feces from 120 to 168 hours; the upper value reflected the addition of dose percentage from this single animal after 120 hours to the group's pre-120-hour value.

^bWhen M17 and M2 are both present in the same matrix, they are indistinguishable in the radio and ion chromatogram as a single peak. Dose percentage values derived from this combined peak were therefore reported.

C_{max} , and bioavailability (F) were also well predicted (Table 5). The fraction absorbed (F_a) was estimated at 67% after a single p.o. dose. For multiple dosing, observed plasma profiles of maribavir after b.i.d. dosing at 10, 30, and 90 mg/kg (20, 60, and

180 mg/kg/day total) were all reasonably characterized by model predictions (Fig. 6, D and E), and median %PE was 17%, 20%, and 29% for 10, 30, and 90 mg/kg b.i.d. regimens, respectively.

Fig. 5. Metabolic pathways of maribavir in cynomolgus monkeys. Italicized letters in parentheses denote matrices where each metabolite was detected, and numbers next to the arrows denote distinct pathways: (1) *N*-dealkylation (to M4, also known as VP44469), (2) oxidation, (3) glucuronide conjugation, (4) deribosylation, (5) dechlorination, (6) glutathione (GSH) conjugation, and (7) amide hydrolysis after GSH conjugation. *b*, bile; *f*, feces; Gluc, glucuronide; *p*, plasma; *u*, urine.

Fig. 6. Observed plasma concentration vs. time profiles of maribavir in cynomolgus monkey overlaid with fitted curves [BDC only (A)] or simulations [intact animals (B, C, D, and E)] in MATLAB SimBiology. (A) Observed data in individual BDC animals overlaid with fitted curve; (B) simulated data with a single intravenous administration in intact animals, using $f_m(\text{Pluc})$ at 0.728 or 0.853 at both 5-mg/kg and 13-mg/kg doses, overlaid with individually observed data; (C) simulated data with a single p.o. administration in intact animals, using $f_m(\text{Pluc})$ at 0.728 at 10-mg/kg dose, overlaid with individually observed data; (D) simulated data with repeated b.i.d. p.o. administration at 10, 30, or 100 mg/kg in intact male animals within the first 8 hours on day 2, using $f_m(\text{Pluc})$ at 0.728, overlaid with individually observed data (Koszalka et al., 2002); (E) simulated data with repeated b.i.d. p.o. administrations at 10, 30, or 100 mg/kg in intact male animals within the first 8 hours on day 27, using $f_m(\text{Pluc})$ at 0.728, overlaid with individually observed data (Koszalka et al., 2002).

Sensitivity Analyses. Important determinants for systemic exposure of maribavir after a single p.o. dose include the overall $f_m(\text{Pluc})$ (Fig. 7A), k_{T_Colon} (Fig. 7B), P_{eff} in the small intestine (Supplemental Fig. 29A; Supplemental Results and Discussion), and Drug_Liver_CL (Supplemental Fig. 29B). The AUC in plasma could be increased by increasing $f_m(\text{Pluc})$, increasing colonic transit time, increasing P_{eff} , or decreasing Drug_Liver_CL . Notably, a change in Drug_Liver_CL would result in little change in the terminal slope; the latter is largely determined by $f_m(\text{Pluc})$, k_{T_Colon} , and, to a lesser degree, P_{eff} . This phenomenon demonstrates the prominent effects of the EHR on systemic exposure of maribavir in monkeys. Systemic C_{max} , on the other hand, is largely driven by P_{eff} and Drug_Liver_CL but not by the other two major parameters. Variations in other parameters, such as small intestine transit time (SITT), rate of hydrolysis of maribavir glucuronides (Gluc_k_hydrolysis), and intercompartmental drug clearance

(Drug_Q12), also lead to changes in plasma exposures of maribavir, but their effects are comparatively minor (Supplemental Fig. 29, C–E).

Discussion

We thoroughly investigated the biotransformation and clearance pathways of maribavir in cynomolgus monkeys by utilizing intact and bile duct-cannulated models and in vitro approaches. We demonstrated that in monkeys, maribavir is primarily metabolized by direct glucuronidation and that these glucuronides, after biliary secretion, can be efficiently converted to maribavir and reabsorbed into circulation. Consequently, in intact animals, maribavir present in gut lumen after intravenous dosing resulted in a higher apparent volume of distribution (V_d) than in BDC animals (Table 2). Conversely, in the BDC group, both the faster clearance due to lack of recirculation and a smaller V_d led to a much shorter

TABLE 5

PK properties observed in in vivo studies with maribavir in cynomolgus monkeys and estimated from the semi-PBPK model. The $f_m(\text{Gluc})$ was set as 0.728 unless specified otherwise. The twice-daily doses were administered 8 hours apart within each dosing day

PK Properties on Maribavir in Plasma	Observed, Mean $\pm$ S.D.	Estimated/Predicted by Semi-PBPK Model
13 mg/kg ^{14}C -Maribavir Intravenous Bolus, Single Dose		
AUC_{0-t} (mg·h/l)	17.4 $\pm$ 3.9	12.5 ($f_m(\text{Gluc}) = 0.728$) 15.1 ($f_m(\text{Gluc}) = 0.853$)
CL (l/h)	2.99 $\pm$ 0.73	4.02 ($f_m(\text{Gluc}) = 0.728$) 3.33 ($f_m(\text{Gluc}) = 0.853$)
V_{ss} (l)	35.4 $\pm$ 6.5	35.2 ($f_m(\text{Gluc}) = 0.728$) 45.8 ($f_m(\text{Gluc}) = 0.853$)
5 mg/kg Maribavir Intravenous Bolus, Single Dose		
AUC_{0-t} (mg·h/l)	4.55 $\pm$ 0.71	5.05
CL (l/h)	4.47 $\pm$ 0.18	4.06
V_{ss} (l)	36.8 $\pm$ 14.7	35.4
10 mg/kg Maribavir Oral Gavage, Single Dose		
AUC_{0-t} (mg·h/l)	6.13 $\pm$ 3.6	6.21
C_{max} (mg/l)	1.50 $\pm$ 1.0	1.01
T_{max} (h)	2 $\pm$ 0	1.31
F	0.661 $\pm$ 0.33	0.615
F_a	N/A	0.671
10 mg/kg Twice-Daily Maribavir Oral Gavage, Multiple Dose		
AUC (mg·h/l), day 2, 0–8 h	3.60 $\pm$ 1.2	4.41
C_{max} (mg/l), day 2, 0–8 h	0.97 $\pm$ 0.39	1.13
AUC (mg·h/l), day 27, 0–8 h	4.60 $\pm$ 0.33	4.83
C_{max} (mg/l), day 27, 0–8 h	0.90 $\pm$ 0.05	1.20
30 mg/kg Twice-Daily Maribavir Oral Gavage, Multiple Dose		
AUC (mg·h/l), day 2, 0–8 h	8.70 $\pm$ 1.2	13.2
C_{max} (mg/l), day 2, 0–8 h	2.55 $\pm$ 0.63	3.40
AUC (mg·h/l), day 27, 0–8 h	12.8 $\pm$ 1.4	14.5
C_{max} (mg/l), day 27, 0–8 h	2.63 $\pm$ 0.38	3.59
90 mg/kg Twice-Daily Maribavir Oral Gavage, Multiple Dose		
AUC (mg·h/l), day 2, 0–8 h	32.1 $\pm$ 11	39.7
C_{max} (mg/l), day 2, 0–8 h	7.3 $\pm$ 3.5	10.2
AUC (mg·h/l), day 27, 0–8 h	30.6 $\pm$ 3.2	43.5
C_{max} (mg/l), day 27, 0–8 h	6.9 $\pm$ 1.1	10.8

AUC_{0-t} , area under the conc.-time curve from time 0 to the last measurable time point; CL, clearance; F, bioavailability; F_a , fraction absorbed; N/A, not available; T_{max} , time to peak conc.; V_{ss} , volume of distribution at steady state.

maribavir $t_{1/2}$ (Fig. 3B; Table 2). A semi-PBPK model was then constructed with data from literature in in vitro and in vivo BDC animals (Supplemental Table 5; Table 1) ; this qualified model captured the pharmacokinetics of maribavir in intact monkeys after either intravenous or oral administration and also identified important compound- and animal-related parameters that impact the kinetics of recirculation.

A previous mass-balance study in intact monkeys (Koszalka et al., 2002) found a large amount of radioactivity in feces as parent and concluded that maribavir was secreted into the bile and subsequently reabsorbed. It may be tempting to claim that the biliary secretion was facilitated through efflux of parent compound at the canalicular surface of hepatocytes (Patel et al., 2016). In this study, we clarified that biliary section of parent drug was actually a minor pathway despite maribavir being a substrate of efflux transporters (Welty et al., 2018; Song et al., 2019a) and that the biliary secretion is primarily facilitated through various glucuronides of maribavir. We assumed that the liver is the primary organ in which glucuronidation of maribavir occurs because of the low percentage of total (%dose) recovered in urine as maribavir glucuronides (Table 4) and because maribavir is an in vitro substrate of human UDP-glucuronosyltransferase (UGT) 1A1, UGT1A3, and UGT2B7 (unpublished data). Additionally, in monkeys the liver expresses the highest levels of UGT1A and UGT2B and displays much stronger glucuronidation activities than do other organs (Albert et al., 2000).

The hydrolysis of glucuronides by the gut microbiome is mediated by β -glucuronidases (GUSs) (Zenser et al., 1999). Bacterial GUSs are

known to metabolize glucuronides, which occurs in the lumen of distal ileum and colon (Zenser et al., 1999; Koppel et al., 2017; Tropini et al., 2017). The degradation rate constant of typical *O*-glucuronides by colonic microbiota was estimated at 3.1 hour⁻¹ in monkeys [derived from (Kim and Jin, 2001; Peters, 2012; Sender et al., 2016); see Supplemental Methods]. Even considering uncertainty related to different *O*-glucuronide substrates, the GUS-mediated hydrolysis of glucuronides is still significantly faster than the typical colon transit rate in monkeys (0.042 hour⁻¹). After biliary secretion likely mediated by transporters (Patel et al., 2016), maribavir glucuronides travel along the GI lumen to the distal ileum and colon, where GUS-expressing bacteria reside. Indeed, between intact and BDC groups, maribavir plasma concentration time courses diverged between 2 and 6 hours postintravenous dose (Fig. 3B), matching SITT around 2.7 hours in monkeys (Table 1) (Ikegami et al., 2003). It is of note that the “second peak” of plasma concentration commonly observed for other enterohepatically recirculated compounds was not apparent in the mass-balance study (Fig. 3) but was evident in another study (Fig. 6B; 5 mg/kg group) at ~8 hours postdose; this may be attributed to different blood collection schedules of the two studies.

Given the abundant knowledge of GI tract physiology and its successful usage for modeling drug absorption (Yu and Amidon, 1999), we created a customized semi-PBPK model to integrate in vivo and in vitro data for maribavir and its metabolites in monkeys. A previous PBPK model (Wu, 2012) provided a theoretical basis on the impact of glucuronide bioconversion to PK of the aglycone, especially on the latter’s

Fig. 7. Sensitivity analyses of effects of (A) $f_m(\text{Gluc})$ and (B) transit rate in the colon lumen (k_{T_Colon}) on pharmacokinetic profile of maribavir after a 10-mg/kg single oral administration to cynomolgus monkeys.

terminal half-life and bioavailability; however, the model did not consider the difference between the small and large intestine (radii, transit time, and location of the gut microbiota), nor were any measured drug or metabolite PK data included to validate the model. In our work, the GI tract was compartmentalized with representations of different physiology within each segment; in particular, the colon was separated into five compartments to represent the spatial and temporal transit of both maribavir and its glucuronides. This compartmentalization is a simplification of the continuous transit model of drug-containing intestinal fluid that requires much more complex mathematical methods, but it is also an improvement over models that do not focus on drug absorption and transit in the colon (Yu and Amidon, 1999; Wu, 2012). As a result, the novel semi-PBPK model is able to characterize the segmental (re)absorption of maribavir after either intravenous or p.o. dosing (Supplemental Table 8). Notably, we did not model drug dissolution because of maribavir's aqueous solubility profiles (0.8 mg/ml in water; 34 and 0.67 mg/ml at pH 3 and 6.6, respectively; unpublished data) and because all dosing formulations were in solution; it was also deemed unnecessary to model metabolism of maribavir within the gut wall because of its high permeability and low cytochrome P450-driven intrinsic clearance.

In the semi-PBPK model, four parameters were fitted from the BDC animals' parent PK profiles, whereas all other parameters were either directly from literature or derived from *in vitro* data or *in vivo* metabolite data from BDC animals. We then demonstrated that this model captured PK profiles in intact monkeys after single intravenous, single p.o., or multiple p.o. administrations. Through sensitivity analyses, we identified important parameters determining the AUC, C_{max} , and terminal elimination rate for maribavir in plasma. The terminal slope is mostly driven by $f_m(\text{Gluc})$ and colon transit rate rather than by hepatic clearance, although the latter still drives the overall exposure of maribavir in terms of AUC and C_{max} . In contrast, SITT and luminal hydrolytic rates of the maribavir glucuronides have much smaller impacts on maribavir

exposure in intact animals (Supplemental Fig. 29). This semi-PBPK model is applicable to other xenobiotics exhibiting similar phenomena, aiding in comprehension and projection of EHR in preclinical/translational settings; in particular, sensitive drug- and species-related parameters should be given the most attention (Fig. 7; Supplemental Fig. 29).

The f_m contributed by each potential clearance pathway is a key drug metabolism and PK metric for small molecular drug candidates because of its implications in the lead optimization process, species translation, clinical study design, and population variability (Di, 2017). For maribavir, it is clear that a higher $f_m(\text{Gluc})$ will lead to more apparent EHR and higher systemic exposure, as more glucuronides will be generated by hydrolysis in the gut lumen and releasing parent drug for reabsorption. The $f_m(\text{Gluc})$ for maribavir in monkey was high at 73% (in vivo) to 85% (hepatocytes) but only around 20% in human hepatocytes. Hence, in theory, humans should demonstrate a less significant EHR compared with monkeys. Indeed, in humans, ^{14}C -maribavir was primarily eliminated through CYP3A-mediated metabolism with renal clearance as a minor pathway; M4 (VP44469) was the principal metabolite observed in urine and feces (Song et al., 2019b), and no direct glucuronides were detected in feces (unpublished data). No apparent recirculation was observed for plasma profiles of maribavir in humans (Wang et al., 2003), and the $t_{1/2}$ observed in humans at around 3.5–7 hours (Wang et al., 2003; Ma et al., 2006; Goldwater et al., 2008; Song et al., 2019a) also mimicked sensitivity analyses wherein $f_m(\text{Gluc})$ was changed to 20% (Fig. 7C), at which scenario the simulated $t_{1/2}$ became ~6 hours (vs. ~15 hours at $f_m(\text{Gluc})$ of 80%). The much lower $f_m(\text{Gluc})$ for maribavir in humans thus confers not only a lower risk of drug-drug interaction for increased systemic concentrations of maribavir and its glucuronides when maribavir is coadministered with inhibitors of canalicular efflux transporters (Zamek-Gliszczynski et al., 2014; Patel et al., 2016) or with inhibitors of UGTs (Zhang et al., 2015) but also less risk of reduced systemic concentrations of maribavir due to

potential induction of UGTs (Court, 2010). In addition, intersubject variabilities in intrinsic factors, such as UGT polymorphism (Court, 2010) and colonic transit time (Vinarov et al., 2021), should have less impact on maribavir exposure in humans due to the lower $f_m(\text{Gluc})$. Therefore, for drugs that undergo EHR through formation and degradation of conjugative metabolites, species differences in metabolism should be considered when extrapolating PK properties from nonclinical species to humans (Kimoto et al., 2017).

In conclusion, using both intact and bile duct-cannulated animals to measure the metabolism and disposition of maribavir, we quantitatively demonstrated that the primary in vivo clearance pathway of maribavir in monkeys was direct glucuronidation. In intact animals, maribavir undergoes enterohepatic recirculation through biliary secretion of maribavir glucuronides followed by hydrolysis of these glucuronides in the gut lumen and subsequent reabsorption of parent. Finally, the overall pharmacokinetics and disposition of maribavir were mechanistically modeled using semi-physiologically based approaches; the novel model captured maribavir's pharmacokinetic and EHR behavior in intact animals and is indicative of lack of EHR in humans when species differences are incorporated.

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Authorship Contributions

Participated in research design: Sun, Welty.

Conducted experiments: Sun.

Contributed new reagents or analytic tools: Sun.

Performed data analysis: Sun.

Wrote or contributed to the writing of the manuscript: Sun, Welty.

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