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

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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-ribofuransyl-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 foscarinet) 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; Papanicolau 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).
After intravenous or oral administration of 14C-labeled maribavir to both intact rat and monkey, a large proportion of radioactivity (≥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). 14C-maribavir (2.43 µCi/mg) and d14-maribavir were provided by Almac Sciences (Craigavon, Northern Ireland, United Kingdom). The chemical purity and radioactive purity of 14C-maribavir (lot SJI-0004E-010-01) were ≥98%. Cryopreserved mixed-sex pooled human hepatocytes (20 donors) and cynomolgus monkey hepatocytes (6 donors) were purchased from BioréclamationIVT (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.** 14C-Maribavir dosing solution was prepared on the day of dosing. Animals were dosed with 13 mg/kg 14C-maribavir (corresponding to a mean 31.5 µCi/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 14C-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. In these animals, 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 canulae 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.
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**Data and Modeling in Cynomolgus Monkeys**

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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.

**Fece**. 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 were injected with a Shimadzu Nexera SIL-30ACMP autoinjector (10°C) equipped with a Promine BCE-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 0.033, 0.05, 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, 8, 24, 36, 48, 72, and 96 hours postdose. Monkey plasma samples added with the internal standard (d-mari- bravir) 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 quadruple 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/concentration) of peak area ratios of the maribavir and internal standard. Additional PK parameters were calculated 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^-4, 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 120-hour pooled – 72-hour pool. Maribavir was incubated in 1 ml at 106 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, microporous, 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 SimBiologYu Simulation 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 (ka) determined by effective permeability (Peffective) specific to each segment (see Supplemental Methods and Supplemental Table 3). Human jejunal Peffecteva 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 Peffecteva (apparent permeability) data (see Supplemental Fig. 2; Supplemental Methods; Supplemental Table 4). Monkey jejunal Peffecteva 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_QCL), 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^-4, 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).

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**In Vitro Metabolism of 14C-Maribavir in Hepatocytes**

The in vitro metabolism of maribavir was evaluated in cryopreserved hepatocytes isolated from cynomolgus monkeys or humans. 14C-Maribavir (10 μM) was incubated in 1 ml at 106 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.
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 1.0e-5; 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):
\[
\%PE = \frac{C_{pred} - C_{obs}}{C_{obs}} \times 100\%
\]
in which 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,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 14C-Maribavir after a Single Intravenous Bolus Injection to Cynomolgus Monkeys**
After a single 13-mg/kg i.v. dose of 14C-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 14C-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 14C-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 (2.71 hours) was about half that of BDC animals (5.72 hours). 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).
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 circulating radioactivity. At all time points, no individual metabolite accounted for more than 8% of circulating radioactivity. For plasma from both intact and BDC animals, the majority (73%) of administered dose was recovered as unchanged parent compound in feces. The dealkylation product M4 itself only accounted for a small fraction (1.3%) of the dose; the 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. 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. 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. 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. 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.

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

**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 ($n = 3$), group 2: BDC monkeys ($n = 3$).

<table>
<thead>
<tr>
<th>PK Parameter on Total Radioactivity in Plasma</th>
<th>Group 1 (Intact), Mean ± S.D.</th>
<th>Group 2 (BDC), Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-t}$ (ng maribavir equivalent·h/g)</td>
<td>28,000 ± 5770</td>
<td>17,100 ± 4050</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng maribavir equivalent·h/g)</td>
<td>30,300 ± 5900</td>
<td>17,900 ± 3470</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>19.7 ± 3.4</td>
<td>2.71 ± 1.4</td>
</tr>
<tr>
<td>CL (g/h)</td>
<td>1700 ± 347</td>
<td>3010 ± 528</td>
</tr>
<tr>
<td>$V_{ss}$ (g)</td>
<td>27,700 ± 3910</td>
<td>6010 ± 1110</td>
</tr>
</tbody>
</table>

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

### Simulations for Intravenous Bolus Dosing of Maribavir in Monkeys
Two fm(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 ¹⁴C-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 fm(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 fm(Gluc) = 0.853 and 49% at fm(Gluc) = 0.728; median %PE for 5 mg/kg i.v. was 81% at fm(Gluc) = 0.853 and 30% at fm(Gluc) = 0.728. The overall median %PE for all available PK data points after intravenous dosing in intact animals was 38% at fm(Gluc) = 0.853 and 1% at fm(Gluc) = 0.728. The fm(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 fm(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,
Cmax, and bioavailability (F) were also well predicted (Table 5). The fraction absorbed (Fa) 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.

### Table 4

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Description</th>
<th>Percentage of Dose Administered to Group 1</th>
<th>Percentage of Dose Administered to Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine (0–144 h)</td>
<td>Feces (0–168 h)</td>
</tr>
<tr>
<td>M15</td>
<td>Loss of chlorine + cysteine conjugate</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M1</td>
<td>Glucuronide conjugate to M4</td>
<td>2.16</td>
<td>ND</td>
</tr>
<tr>
<td>M7</td>
<td>Glucuronide conjugate to M4</td>
<td>2.62</td>
<td>ND</td>
</tr>
<tr>
<td>M16</td>
<td>Glucuronide conjugate to M4</td>
<td>0.297</td>
<td>ND</td>
</tr>
<tr>
<td>M17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Glucuronide conjugate to M4</td>
<td>0–1.85</td>
<td>ND</td>
</tr>
<tr>
<td>M2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Loss of ribose + glucuronide</td>
<td>0–1.85</td>
<td>ND</td>
</tr>
<tr>
<td>M10</td>
<td>Glucuronide conjugate to parent</td>
<td>0.402</td>
<td>ND</td>
</tr>
<tr>
<td>M4</td>
<td>Loss of propyl</td>
<td>2.0</td>
<td>8.80–9.34</td>
</tr>
<tr>
<td>M5</td>
<td>Oxidation on propyl</td>
<td>ND</td>
<td>0.416–0.433</td>
</tr>
<tr>
<td>M11</td>
<td>Glucuronide conjugate to parent</td>
<td>1.2</td>
<td>ND</td>
</tr>
<tr>
<td>M12</td>
<td>Glucuronide conjugate to parent</td>
<td>2.01</td>
<td>ND</td>
</tr>
<tr>
<td>Maribavir</td>
<td>Parent</td>
<td>0.823</td>
<td>57.9–60.3</td>
</tr>
</tbody>
</table>

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

<sup>a</sup>The 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.

<sup>b</sup>When 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.

---

**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.
Sensitivity Analyses. Important determinants for systemic exposure of maribavir after a single p.o. dose include the overall \( fm(Gluc) \) (Fig. 7A), \( k_T_Colon \) (Fig. 7B), \( Peff \) 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 \( fm(Gluc) \), increasing colonic transit time, increasing \( Peff \), 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 \( fm(Gluc) \), \( k_T_Colon \), and, to a lesser degree, \( Peff \). 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 \( Peff \) 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 (Vd) than in BDC animals (Table 2). Conversely, in the BDC group, both the faster clearance due to lack of recirculation and a smaller Vd led to a much shorter
maribavir t_{1/2} (Fig. 3B; Table 2). A semi-PBPK model was then constructed with data from literature 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 being a substrate of efflux transporters (Welty et al., 2018; Song et al., 2019a) and that the biliary secretion is primarily facilitated through various 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

### Table 5

<table>
<thead>
<tr>
<th>PK Properties on Maribavir in Plasma</th>
<th>Observed, Mean ± S.D.</th>
<th>Estimated/Predicted by Semi-PBPK Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>13 mg/kg 14C-Maribavir Intravenous Bolus, Single Dose</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-1} (mg·h/l)</td>
<td>17.4 ± 3.9</td>
<td>12.5 (t_{1/2} (Gluc) = 0.728)</td>
</tr>
<tr>
<td>CL (l/h)</td>
<td>2.99 ± 0.73</td>
<td>15.1 (t_{1/2} (Gluc) = 0.853)</td>
</tr>
<tr>
<td>V_{ss} (l)</td>
<td>35.4 ± 6.5</td>
<td>4.02 (t_{1/2} (Gluc) = 0.728)</td>
</tr>
</tbody>
</table>

| **5 mg/kg Maribavir Intravenous Bolus, Single Dose** | | |
| AUC_{0-1} (mg·h/l) | 4.55 ± 0.71 | 5.05 |
| CL (l/h) | 4.47 ± 0.18 | 4.06 |
| V_{ss} (l) | 36.8 ± 14.7 | 35.4 |

| **10 mg/kg Maribavir Oral Gavage, Single Dose** | | |
| AUC_{0-1} (mg·h/l) | 6.13 ± 3.6 | 6.21 |
| C_{max} (mg/l) | 1.50 ± 1.0 | 1.01 |
| t_{max} (h) | 2 ± 0 | 1.31 |
| F | 0.661 ± 0.33 | 0.615 |
| Fa | N/A | 0.615 |

| **10 mg/kg Twice-Daily Maribavir Oral Gavage, Multiple Dose** | | |
| AUC (mg·h/l), day 2, 0–8 h | 3.60 ± 1.2 | 4.41 |
| C_{max} (mg/l), day 2, 0–8 h | 0.97 ± 0.39 | 1.13 |
| AUC (mg·h/l), day 27, 0–8 h | 4.60 ± 0.33 | 4.83 |
| C_{max} (mg/l), day 27, 0–8 h | 0.90 ± 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 ± 1.2 | 13.2 |
| C_{max} (mg/l), day 2, 0–8 h | 2.55 ± 0.63 | 3.40 |
| AUC (mg·h/l), day 27, 0–8 h | 12.8 ± 1.4 | 14.5 |
| C_{max} (mg/l), day 27, 0–8 h | 2.63 ± 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 ± 11 | 39.7 |
| C_{max} (mg/l), day 2, 0–8 h | 7.3 ± 3.5 | 10.2 |
| AUC (mg·h/l), day 27, 0–8 h | 30.6 ± 3.2 | 43.5 |
| C_{max} (mg/l), day 27, 0–8 h | 6.9 ± 1.1 | 10.8 |

AUC_{0-1}, area under the conc.-time curve from time 0 to the last measurable time point; CL, clearance; F, bioavailability; Fa, fraction absorbed; N/A, not available; T_{max}, time to peak conc.; V_{ss}, volume of distribution at steady state.
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\text{max} and terminal elimination rate for maribavir in plasma. The terminal slope is mostly driven by f_m(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\text{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(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(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, 14C-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\text{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(Gluc) was changed to 20% (Fig. 7C), at which scenario the simulated t\text{1/2} became ~6 hours (vs. ~15 hours at f_m(Gluc) of 80%). The much lower f_m(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

![Fig. 7. Sensitivity analyses of effects of (A) f_m(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.](image-url)
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 \( t_{\text{m}} \text{Gluc} \). 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. We 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.

References


Address correspondence to: Kefeng Sun, 300 Shire Way, Lexington, MA 02421. E-mail: kefeng.sun@takeda.com
Supplemental Material

**Title:** Elucidation of Metabolic and Disposition Pathways for Maribavir in Non-human Primates Through Mass Balance and Semi-Physiologically Based Modeling Approaches

**Authors:** Kefeng Sun and Devin Welty

**Journal:** Drug Metabolism and Disposition

**Manuscript number:** DMD-AR-2021-000493

**Methods**

**Animal Preparation, BDC Surgery and Dosing**

**Animal Preparation.** Certified Primate Diet #5048 was provided to animals unless otherwise specified under surgical and clinical pathology procedures. Diets were supplemented with appropriate fruits and cereals. Water was provided fresh daily, ad libitum. During acclimation, animals were housed in stainless steel cages and provided with cage enrichment devices and treats. Enrichment devices and treats were withheld from the time of dose administration until at least 96 hours after dosing to ensure no radioactivity was ingested from contaminated sources.

The animal room was maintained at a temperature of 20–26 °C, relative humidity of 50 ± 20%, and with a 12-hour light/dark cycle. Dark cycles were interrupted to accommodate study procedures as necessary.

Prior to bile-duct cannulation (BDC) surgery, animals were acclimated to the jacket and tether system for bile collection. Immediately after surgery, a jacket was placed on each animal and the catheters were guided through a tether. Antibiotics, analgesics, and intravenous (i.v.) fluids were administered as deemed appropriate by a staff veterinarian.

Animals received 5% dextrose solution and Lactated Ringer’s solution via the duodenal cannula continuously through 0–3 days and 3–7 days post-surgery, respectively. Mixed bile salts replacements were administered to animals from 7 days post-surgery to sacrifice.
**BDC Surgery.** A pre-surgery physical examination was conducted on each animal by a veterinarian. Animals were fasted overnight before surgery. An anesthetic regimen of appropriate medications for sedation and inhaled anesthetic for maintenance was used. Using sterile surgical procedures, the bile duct was cannulated to allow collection of bile, and a second cannula was placed into the duodenum to allow infusion of a bile salts replacement solution or other fluids, as required. Immediately after surgery, a jacket was placed on each animal and the catheters were guided through a tether. Antibiotics, analgesics, and i.v. fluids were administered as deemed appropriate by a veterinarian. Animals were permitted to recover for 10–13 days after surgery.

**Dosing.** On the day of dosing, 432.2 mg of $^{14}$C-maribavir was combined with 4.9 mL of ethanol and mixed. Then, 10.5 mL of propylene glycol was added and the formulation mixed. Finally, 54.6 mL of saline was added and the formulation was magnetically stirred for 15 minutes.

The volume of radiolabeled dose formulation administered to each animal was calculated based on the body weight (minus jacket weight as appropriate) taken on the day of dose administration. The actual amount administered was determined by weighing the dose syringe before and after dose administration. Stability of the radiolabeled maribavir under conditions of administration was demonstrated by analyzing pre-dose and post-dose aliquots by radio-HPLC. Confirmation of stability of the dose formulation under the conditions of administration (column recovery: 102% and 101% for pre- and post-dose aliquots, respectively) serves as confirmation of stability of the test article used to formulate the dose.

**Pharmacokinetics and Excretion Balance**

**Sample Collection.** Samples for peripheral blood mononuclear cell isolation were pooled into sodium nitrate cell preparation tubes. Tubes were inverted gently 3–4 times and stored upright at ambient temperature before being processed. Centrifugation was performed as soon as possible following collection.

**Plasma.** Blood collected for radioanalysis and metabolite profiling was collected into tubes containing K$_2$-ethylenediaminetetraacetic acid (K$_2$EDTA) and maintained in chilled cryoracks until aliquoted for radioanalysis. The remaining sample was stored at −70 °C for metabolite profiling. Blood samples obtained for plasma derivation were maintained in chilled cryoracks until
centrifuged to obtain plasma. A 200 µL aliquot was placed into a tube and retained for bioanalysis. The remaining plasma was retained for radioanalysis and metabolite profiling; cellular fractions were discarded. Samples were stored at −70 °C until radioanalysis.

**Urine.** Urine was collected in plastic containers surrounded by dry ice and the weight of each sample was recorded. Any urine excreted outside of the cage was collected with gauze and saved for radioanalysis.

**Feces.** Feces were collected at ambient temperature in plastic containers.

Bile collected from BDC animals was collected into plastic containers surrounded by dry ice. The weight of each sample was recorded.

**Cage Wash/Cage Wipe.** After each 24-hour excreta collection through to 312 hours post-dose, cages were rinsed with water. Cage rinse samples were collected into plastic containers, and the weight of each sample was recorded. Cage debris, consisting mainly of hair and food, was collected daily and pooled by animal. After the last excreta collections, cages were washed and wiped with a solution of 1% trisodium phosphate in water. The cage wash and cage wipe samples were collected into separate plastic containers, and the weight of each cage wash sample was recorded.

**Radioactivity Measurement.** Double aliquots of all samples were analyzed for radioactivity concentrations. Each sample was homogenized before analysis unless the entire sample was used. All samples were analyzed in duplicate if sample size allowed; if results from duplicates differed by more than 10% from the mean value, the sample was re-homogenized and reanalyzed.

Blood samples were digested with solubilizing agent before incubation with 0.1 M disodium salt of ethylenediaminetetraacetic acid (Na₂EDTA) and 30% hydrogen peroxide. Resultant samples were then left overnight and scintillation cocktail was added before analysis by liquid scintillation counters (LSC). Fecal samples were homogenized in solvent (reverse osmosis water:acetonitrile [ACN]; 60:40) and digested with sodium hydroxide before analysis by LSC.
**Metabolite Profiling**

**Radioactivity Extraction Recovery.** Extraction recoveries for each excreta was determined by combining samples with acetonitrile and the supernatant of sonicated, vortex-mixed, centrifuged samples removed. The extraction was repeated and the respective supernatants were combined. Duplicate aliquots were analyzed by LSC to determine the percentage of radioactivity extracted. The eluents for representative urine, feces, and bile samples were determined for the column recoveries, and ranged from 97.4 to 107%.

**Plasma Samples.** Plasma samples obtained from male monkeys in Groups 1 and 2 at 0.083, 0.25, 0.5, 2, 6, 24 (Group 1 only), and 48 (Group 1 only) hours post-dose were pooled by group and time point to generate seven (Group 1) and five (Group 2) pooled samples, including 0.06 to 0.15 g of each sample by weight. The radioactivity in each pooled sample was determined by LSC. Two additional plasma samples (Group 1, Animal #3, 72-hour and Group 2, Animal #3, 24-hour) were processed following the same procedures as the pooled samples. An aliquot of each plasma pool or individual sample was combined with acetonitrile, centrifuged, and the supernatant removed. The extraction was repeated and the respective supernatants combined. Combined supernatants were evaporated to dryness under nitrogen and reconstituted in reverse osmosis water (300 µL).

**Urine samples.** Thirteen pooled urine samples were generated for Group 1: a pooled 0–48-hour sample (from 0–8, 8–24, and 28–48 hours post-dose) and samples pooled by collection interval at 48–72, 72–96, 96–120, 120–144, 144–168, 168–192, 192–216, 216–240, 240–264, 264–288, 288–312, and 312–336 hours post-dose. Seven pooled urine samples were generated for Group 2: a pooled 0–24-hour sample (from 0–8 and 8–24 hours post-dose) and samples pooled by collection interval at 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours post-dose.

The Group 1 72–96, 96–120, and 120–144-hour samples and the Group 2 24–48-hour pooled samples were concentrated.

**Feces Samples.** Approximately 2 g of each sample was combined with acetonitrile, sonicated, vortex mixed, centrifuged, and the supernatant collected. The extraction was repeated and the respective supernatants combined. Combined supernatants were evaporated to dryness under
nitrogen and reconstituted in 300 μL of methanol (MeOH). Due to low recoveries, Group 2 24–48- and 48–72-hour samples were reconstituted in an additional 100 μL of MeOH.

**LC-MS/MS Instrumentation and Conditions.** Details of LC-MS/MS setup for metabolite profiling are listed below.

**LC-MS instrumentation for metabolite profiling:**

<table>
<thead>
<tr>
<th>Controller:</th>
<th>Shimadzu/Prominence CBM-20A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumps:</td>
<td>Shimadzu/Nexera LC-30AD</td>
</tr>
<tr>
<td>Autoinjector:</td>
<td>Shimadzu/Nexera SIL-30ACMP (10 °C)</td>
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<tr>
<td>Column oven:</td>
<td>Shimadzu/Prominence CTO-20AC (45 °C)</td>
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<tr>
<td>Degasser:</td>
<td>Shimadzu/Prominence DGU-20A5R</td>
</tr>
<tr>
<td>Mass spectrometer:</td>
<td>Thermo Fisher Scientific Q Exactive</td>
</tr>
<tr>
<td>Fraction collector:</td>
<td>Leap Technologies PAL HTC-xt</td>
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**LC-MS conditions for metabolite profiling:**

<table>
<thead>
<tr>
<th>Ionization interface:</th>
<th>Positive/negative heated electrospray interface (HESI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC column:</td>
<td>Waters Atlantis T3, 4.6 x 250 mm, 5 μm</td>
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<tr>
<td>Guard column:</td>
<td>Phenomenex C18, 3 x 4 mm</td>
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<tr>
<td>Mobile phase A:</td>
<td>0.1% formic acid in water</td>
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<tr>
<td>Mobile phase B:</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>Gradient:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time (minutes)</td>
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LC-MS/MS to Determine Maribavir Concentration

For calibration of liquid chromatography–tandem mass spectrometry (LC-MS/MS), nine non-zero calibration standards (1–1,000 ng/mL) and quality control samples (3, 32, 750, and 5,000 ng/mL) were prepared, using cynomolgus monkey plasma that was free of significant interference (BioIVT, Hicksville, NY, USA), and stored at −80 °C. All samples for a given subject were analyzed together in a single batch except when samples had to be re-assayed. A batch, at a minimum, consisted of ≥2 control blanks (control matrix with no internal standard) equal to at least 2% of the unknown samples, 2 standard zero samples (control matrix with internal standard only), and 1 replicate of at least 6 different calibration standards (non-zero standards); replicate low, medium, and high concentration QC samples were also included to reflect at least 5% of the number of unknown samples (minimum n = 2 QC samples per concentration level). Calibration curves and standard curves were created based on linear regression. The batch acceptance criteria were: (1) standards were rejected if they were greater than ±15% (all standards but the LLOQ) or ±20% (LLOQ only) of the nominal concentration; (2) at least 75% of the non-zero standards were within the respective acceptance criterion; and (3) at least two-thirds of the low, medium, and high QCs, including at least 50% at each concentration, were valid data points and were within ±15% of the nominal concentration. In terms of between-batch precision and accuracy, the percent coefficient of variation (%CV) for the 3, 32, 750, and 5,000 ng/mL QC samples were 3.3%, 1.1%, 2.6%, and 1.6%, and the percent bias was −7.7%, −8.4%, −10.8%, and −12.4% for the QC samples at the 4 respective concentrations. The percent bias for the standard curve samples ranged from −4.5% to 6.6%.

Metabolism of 14C-maribavir in Hepatocytes

Cryopreserved cynomolgus monkey or human hepatocytes were thawed in a 37 °C water bath with gentle shaking and suspensions were immediately transferred to a centrifuge tube containing pre-warmed media. The suspension was centrifuged at 50 G for 5 minutes. After centrifugation, the supernatant was removed, pre-warmed incubation media added, and the hepatocytes resuspended. Cell viability was >70%, determined by Trypan Blue staining. 14C-maribavir (10 μM) was incubated in 10⁶ cells/mL at 37 °C for 4 hours and ACN then added. After centrifugation, the
supernatant was transferred, dried, and reconstituted in 25% ACN in water. Aliquots were analyzed by LC/UV/MS and LC/UV/radioactivity, using an Agilent 1100 high-performance liquid chromatography (quaternary pumps, autosampler, and diode array UV detector), a Linear Trap Quadropole (LTQ) Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA), and a PerkinElmer 625TR radioactivity flow detector. The radioactivity flow detector equipped with a 200 μL flow cell was operated using scintillation cocktail (Ultima Fio M) at flow rate of 1.2 mL/min. Separation was achieved on a Phenomenex Luna C18 (2) column (2.0 × 250 mm, 5 μm) (Torrance, CA, USA). The mass spectrometer was operated in electrospray positive (ES+) ionization mode. Mass spectra were acquired in full scan (m/z 150 to 1,500) and data-dependent scan (MS² and MS³) modes. The radioactivity in extracts of hepatocyte incubations was determined by liquid scintillation analysis of aliquots of the extracts.

**Permeability of Maribavir Across Caco-2 Cell Monolayer**

The permeability assay buffer was Hank’s Balanced Salt solution containing 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 15 mM glucose at a pH of 7.0–7.2; the receiver side contained 1% bovine serum albumin. The dosing solution concentration contained 10 μM compounds in assay buffer. Compounds tested were maribavir, atenolol (as low permeability control), and propranolol (as high permeability control). Cells were dosed, in duplicates, on the apical side (apical-to-basolateral) or basolateral side (basolateral-to-apical) and incubated at 37 °C with 5% carbon dioxide in a humidified incubator. At 1 and 2 hours, a 200 μL aliquot was taken from the receiver chamber and replaced with fresh assay buffer. Lucifer Yellow permeation was also measured for each monolayer after being subjected to the test article to confirm monolayer integrity. Concentrations of test article were determined by LC/MS in ES+ mode. The apparent permeability ($P_{app}$) for each compound was calculated using Supplemental Equation 1:

$$P_{app} = \frac{dC_R}{dt} \cdot \frac{V_R}{A \cdot C_0}$$

Supplemental Equation 1

where $dC_R/dt$ is the slope for concentrations in the receiving chamber over time, $V_R$ is the volume of the receiving chamber, $A$ is the area for the monolayer, and $C_0$ is the initial concentration in the donor chamber.
Calculation of Intestinal Effective Permeability from Apparent Permeability. Linear regression analyses were first performed with natural logs (Ln) of human effective permeability ($P_{\text{eff}}$) to Ln(Caco-2 $P_{\text{app}}$) on reference compounds using one set of human $P_{\text{eff}}$ data (Larregieu and Benet, 2013) and two sets of Caco-2 $P_{\text{app}}$ data (Alsenz et al., 2003; Li et al., 2007; Supplemental Table 4). The regression graphs are shown in Supplemental Fig. 2. The equation for linear regression is:

$$y = a \cdot x + b$$

where $y$ is Ln(human $P_{\text{eff}} \times 10^{-4}$ cm/s) and $x$ is Ln(Caco-2 $P_{\text{app}} \times 10^{-6}$ cm/s); $a$ is the coefficient on $x$ and $b$ is the intercept of the regression line when $x = 0$.

After averaging the two $a$ and $b$ values from both datasets (Supplemental Fig. 2), the linear relationship becomes:

$$y = 0.645 \cdot x - 1.642$$

Before plugging in Ln(Caco-2 $P_{\text{app}} \times 10^{-6}$ cm/s) of maribavir into $x$ to project $y$, it has to be adjusted based on differences in the measured $P_{\text{app}}$ data for propranolol and atenolol (Supplemental Table 4, rightmost column):

$$\text{Adjusted Ln}(P_{\text{app}}) = \text{Original Ln}(P_{\text{app}}) + \text{Average(Difference in Ln}(P_{\text{app}}) \text{for reference compounds)}$$

The Ln(Caco-2 $P_{\text{app}} \times 10^{-6}$ cm/s) for maribavir changed to 3.00 from 1.775 after adjustment with $P_{\text{app}}$ data of propranolol and atenolol to previous studies. With $x = 3.00$ in Supplemental Eq. 2, $y$ is calculated to be 0.264; the projected $P_{\text{eff}}$ ($\times 10^{-4}$ cm/s) is, therefore, $e^{0.264} = 1.30$. The $P_{\text{eff}}$ in monkey is assumed to be the same as in human at $1.30 \times 10^{-4}$ cm/s.

Calculation of Segmental Permeability in the Gastrointestinal Tract. The first-order absorption rate ($k_a$) in the jejunum was calculated at 2.34 h$^{-1}$ with:

$$\text{Drug}_{\text{ka}}_{\text{Jejunum}} = 2 \cdot P_{\text{eff}} / \text{Radius}$$

where the radius for cynomolgus monkey jejunum is 0.4 cm (Sugano et al., 2012).
According to Supplemental Eq. 5, the $k_a$ at different segments of the gastrointestinal (GI) tract will be determined by the regional $P_{eff}$ as well as the radius. The regional permeability in duodenum, ileum, and colon could be adjusted from the jejunal permeability using a surface area expansion factor (SAEF; Supplemental Table 3, from Olivares-Morales et al., 2015):

$$Drug_{ka_GI\_region} = Drug_{ka\_jejunal} \cdot \frac{SAEF(GI\_region)}{SAEF(Jejunal)}$$

Supplemental Equation 6

As no information on radii of different regions of the cynomolgus monkey GI tract is readily available, the ratios of radii in duodenum, ileum, and colon to that of the jejunum in monkeys were assumed to be the same as those in humans. The information on SAEF and radii are listed in Supplemental Table 3. The order of the calculated $k_a$ in the four regions of the GI tract is $Drug_{ka\_jejunal} > Drug_{ka\_ileum} > Drug_{ka\_Duodenum} >> Drug_{ka\_Colon}$.

**Calculation of Hydrolysis Rate of Typical O-glucuronide by Bacteria Expressing β-glucuronidases.** The activity of β-glucuronidase (GUS) from healthy human fecal samples with 1 mM substrate incubation has been reported to be approximately 2.6 μmol/min/(g dry weight of bacteria) (Kim et al., 2001). The intrinsic clearance is, therefore, 2.6 μmol/min/g / 1 mM = 2.6 mL/min/(g dry weight of bacteria). The total dry weight of bacteria in humans has been reported to be around 50 g from a healthy 70 kg human (Sender et al., 2016). Assuming similarity between cynomolgus monkey and human for bacterial activity on a per-kg basis, the activity of bacterial GUS in a 4 kg monkey was calculated at 2.6 mL/min/(g dry weight of bacteria) × 50 (g dry weight bacteria) / 70 kg × 4 kg = 7.4 mL/min. If these bacteria are evenly distributed within the luminal space of the colon, given the colonic luminal volume of 146 mL in monkey (Peters et al., 2012), the degradation rate for typical O-glucuronides is thus 7.4 mL/min / 146 mL = 0.051 min$^{-1}$ or 3.1 h$^{-1}$.

**Semi-Physiologically Based Pharmacokinetic Model for Disposition of Maribavir in Monkeys**

The semi-physiologically based pharmacokinetic (semi-PBPK) model comprises a modified compartmental absorption and transit (CAT) module for the GI tract (Yu and Amidon, 1999) with five colon luminal compartments plus three systemic compartments (peripheral, central, and liver). The observed plasma concentrations were assumed to resemble those of the central
The CAT GI model consists of 13 total luminal compartments: one for the stomach, one for the duodenum, two for the jejunum, four for the ileum, and five for the colon. The transit of maribavir or maribavir glucuronides through the GI tract is modeled by small intestine and colon transit rates. The intact versus BDC mode in i.v. dosing was controlled by enabling (BDC_On = 0) or disabling (BDC_On = 1) transit from the duodenum to jejunum. Within each GI luminal segment, maribavir is absorbed with first-order kinetics, with the rate \( k_a \) determined by \( P_{eff} \).

The liver compartment was necessary to model the first-pass metabolism of maribavir after absorption from the gut, as well as the metabolic and biliary elimination of maribavir as glucuronides or others. The Drug_Liver_CL parameter is used to describe the clearance of the drug from the liver compartment. The formation of maribavir glucuronides in the duodenal lumen through hepatic glucuronidation and biliary secretion was assumed to be a first-order process and driven primarily by the formation rate of glucuronides; another module that incorporated the periodic waves of bile flow was initially developed and evaluated, but was found to not improve the predictability of the systemic concentration profile and was ultimately not included in the final model. Luminal conversion of maribavir glucuronides to maribavir was assumed to occur in the distal small intestine and throughout the colon, and that the entirety of regenerated maribavir is available for (re-)absorption. The minor renal excretion pathway was modeled as a first-order elimination process from the central compartment. The fraction absorbed (\( F_a \)) for maribavir after oral dosing is calculated with:

\[
F_a = 1 - \frac{\text{Drug}_\text{Fecal}}{\text{Dose}}
\]

Supplemental Equation 7

Where \( \text{Drug}_\text{Fecal} \) is the simulated amount of maribavir in the feces after a substantial amount of time (≥10 days) post-oral dosing.

To simulate the fraction absorbed from each GI segment, the absorption fluxes (Supplemental Table 1 and Supplemental Fig. 1) were turned off in a stepwise fashion from the last segment of the colon to the duodenum, and the incremental AUC change for each GI segment was recorded. The \( F_a \) from each GI segment is calculated by dividing the incremental AUC change from each segment with the total AUC (when all absorption fluxes were enabled).
The volume of distribution at steady state ($V_{ss}$) for intravenous bolus dosing in intact animals is calculated with:

$$V_{ss} = \frac{Dose \cdot AUMC}{AUC^2} = \frac{Dose \cdot \int_0^\infty (C_p \cdot t) \, dt}{(\int_0^\infty C_p \, dt)^2}$$  

Supplemental Equation 8

Where $C_p$ denotes plasma concentration of maribavir and AUMC is the area under the plasma concentration first moment versus time curve.

**Results and Discussion**

**Parameter Estimation in the Semi-PBPK Model**

**Drug clearance from the liver compartment (Drug_Liver_CL).** The Drug_Liver_CL parameter, estimated at 16 L/h, denotes the clearance of maribavir from the liver compartment. This is different from the conventional drug clearance concept in the plasma compartment (CL$_{plasma}$). An approximate conversion between Drug_Liver_CL and CL$_{plasma}$ can be performed using the well-stirred model (Supplemental Equation 9; assuming negligible non-hepatic clearance pathways):

$$CL_{plasma} = \frac{Q_h \cdot Drug\_Liver\_CL}{Q_h + Drug\_Liver\_CL}$$  

Supplemental Equation 9

Where $Q_h$ is the hepatic blood flow at 2.6 L/h/kg or 9.2 L/h (from 2.6 L/h/kg $\times$ 4 kg body weight) in the mass balance study of maribavir in cynomolgus monkeys (Supplemental Table 5).

When using the $Q_h$ and Drug_Liver_CL values of 9.6 L/h and 16 L/h, respectively, in Supplemental Equation 9, the drug clearance from the plasma compartment was calculated as 6.0 L/h; this was similar to the plasma clearance of maribavir calculated separately from non-compartmental analysis (NCA) of the plasma PK time course (5.72 L/h) for BDC animals.

**Additional Sensitivity Analyses on Important Parameters in the Semi-PBPK Model**

**Intestinal permeability ($P_{eff}$).** For rapidly absorbed drugs that do not undergo enterohepatic recirculation, changes to $P_{eff}$ only affect the absorption phase after oral dosing ($C_{max}$, $T_{max}$) and the
AUC; it typically does not impact the terminal slope of the PK time course. For a drug that displays prominent EHR behavior (e.g., maribavir in monkeys), the terminal PK slope is shallower for a parent drug with high P_{eff}, and steeper if the parent drug has a lower P_{eff} (Supplemental Fig. 29A).

**Drug clearance from the liver (Drug\_Liver\_CL).** For a rapidly absorbed drug that does not undergo enterohepatic recirculation, the terminal elimination phase of the PK time course is in part driven by the systemic clearance of the drug; changing the plasma clearance while fixing other PK parameters would result in changes to the terminal slope of the PK curve. However, for a drug that displays prominent EHR behavior (e.g., maribavir in monkeys), altering the drug clearance in the liver (affecting the nominal drug clearance in plasma; Supplemental Equation 9) would not result in a change to the terminal phase of the plasma PK time course (Supplemental Fig. 29B). Nonetheless, overall exposure (AUC) is still impacted by Drug\_Liver\_CL.

**Small intestine transit time (SITT).** A change to SITT had no impact on the C_{max} of maribavir in monkeys after oral dosing and did not significantly impact the AUC of the parent drug, although faster small intestinal transit was simulated to result in a slightly lower AUC (Supplemental Fig. 29C).

**Rate of hydrolysis of maribavir glucuronides (Gluc\_k\_hydrolysis).** Variation of Gluc\_k\_hydrolysis across a wide range of values had no significant effect on the exposure to maribavir after oral dosing (Supplemental Fig. 29D).

**Intercompartmental drug clearance between the central and peripheral compartments (Drug\_Q12).** Variation of the Drug\_Q12 also had little effect on the exposure to maribavir after oral dosing (Supplemental Fig. 29E).
SUPPLEMENTAL TABLE 1

All fluxes used in the semi-physiologically based pharmacokinetic model for maribavir in cynomolgus monkeys.

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<th>Expression in SimBiology®</th>
<th>Description</th>
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</thead>
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<tr>
<td>Absorption_1</td>
<td>Drug_ka_Duodenum<em>Drug_SI1</em>(1-BDC_On)</td>
<td>Flux of parent from duodenal lumen to liver</td>
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<tr>
<td>Absorption_2</td>
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<td>Flux of parent from jejunal lumen 1 to liver</td>
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<td>Absorption_3</td>
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<td>Absorption_4</td>
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<td>Absorption_6</td>
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<td>Flux of parent from ileal lumen 3 to liver</td>
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<td>Absorption_7</td>
<td>Drug_ka_Ileum*Drug_SI7</td>
<td>Flux of parent from ileal lumen 4 to liver</td>
</tr>
<tr>
<td>Absorption_Colon1</td>
<td>Drug_ka_Colon*Drug_Colon1</td>
<td>Flux of parent from colon lumen 1 to liver</td>
</tr>
<tr>
<td>Absorption_Colon2</td>
<td>Drug_ka_Colon*Drug_Colon2</td>
<td>Flux of parent from colon lumen 2 to liver</td>
</tr>
<tr>
<td>Absorption_Colon3</td>
<td>Drug_ka_Colon*Drug_Colon3</td>
<td>Flux of parent from colon lumen 3 to liver</td>
</tr>
<tr>
<td>Absorption_Colon4</td>
<td>Drug_ka_Colon*Drug_Colon4</td>
<td>Flux of parent from colon lumen 4 to liver</td>
</tr>
<tr>
<td>Absorption_Colon5</td>
<td>Drug_ka_Colon*Drug_Colon5</td>
<td>Flux of parent from colon lumen 5 to liver</td>
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<tr>
<td>Biliary_secretion_liver</td>
<td>(Drug_Liver_ke_Biliary*Drug_Liver)*Liver</td>
<td>Flux of parent from liver to gastrointestinal lumen through biliary excretion</td>
</tr>
<tr>
<td>CL_R</td>
<td>(Drug_ke_renal*Drug_Central)*Central</td>
<td>Flux of parent from central to excreta through renal excretion</td>
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<td>Drug_ka_IV</td>
<td>k_IV_infuse*Dose_IV</td>
<td>Flux of parent during intravenous injection</td>
</tr>
<tr>
<td>Drug_Liver_Met1</td>
<td>(Drug_Liver_ke_met1*Drug_Liver)*Liver</td>
<td>Flux of parent from liver to excreta through non-glucuronidation metabolism to metabolites</td>
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<tr>
<td>Drug_Stomach_Transit</td>
<td>k_T_Stomach*Dose_PO</td>
<td>Flux of parent from stomach lumen to duodenum</td>
</tr>
<tr>
<td>Drug_Tran_Colon1</td>
<td>k_T_Colon_5Cpt*Drug_Colon1</td>
<td>Flux of parent from colon lumen 1 to colon lumen 2</td>
</tr>
<tr>
<td>Description</td>
<td>Formula</td>
<td>Description</td>
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<td>Flux of parent from colon lumen 2 to colon lumen 3</td>
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<td>Flux of parent from colon lumen 3 to colon lumen 4</td>
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<td>Flux of parent from colon lumen 5 to feces</td>
<td>$\text{Drug_Tran_Colon5} = k_T_Colon_5Cpt*\text{Drug_Colon5}$</td>
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<td>Flux of parent from duodenal lumen to jejunal lumen 1</td>
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<tr>
<td>Flux of parent from jejunal lumen 1 to jejunal lumen 2</td>
<td>$\text{Drug_Transit_SI_2} = k_T_Jej_2Cpt*\text{Drug_SI2}$</td>
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<td>Flux of parent from jejunal lumen 2 to ileal lumen 1</td>
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<tr>
<td>Flux of parent from ileal lumen 1 to ileal lumen 2</td>
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<td>Flux of parent from ileal lumen 2 to ileal lumen 3</td>
<td>$\text{Drug_Transit_SI_5} = k_T_Ile_4Cpt*\text{Drug_SI5}$</td>
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<tr>
<td>Flux of parent from ileal lumen 3 to ileal lumen 4</td>
<td>$\text{Drug_Transit_SI_6} = k_T_Ile_4Cpt*\text{Drug_SI6}$</td>
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<tr>
<td>Flux of parent from ileal lumen 4 to colon lumen 1</td>
<td>$\text{Drug_Transit_SI_7} = k_T_Ile_4Cpt*\text{Drug_SI7}$</td>
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<tr>
<td>Flux of parent from liver to duodenum through glucuronidation to glucuronides</td>
<td>$\text{Gluc_from_Liver} = (\text{Drug_Liver_ke_gluc})*\text{Drug_Liver} *\text{Liver}$</td>
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<tr>
<td>Flux of parent from central to excreta through glucuronidation to glucuronides</td>
<td>$\text{Gluc_exc} = (\text{Drug_ke_gluc_exc})*\text{Drug_Central} *\text{Central}$</td>
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<tr>
<td>Flux of glucuronides from duodenal lumen to jejunal lumen 1</td>
<td>$\text{Gluc_SI_Transit_1} = k_T_Duod*\text{Gluc_SI1},(1-BDC_On)$</td>
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<tr>
<td>Flux of glucuronides from jejunal lumen 1 to jejunal lumen 2</td>
<td>$\text{Gluc_SI_Transit_2} = k_T_Jej_2Cpt*\text{Gluc_SI2}$</td>
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<td>Flux of glucuronides from jejunal lumen 2 to ileal lumen 1</td>
<td>$\text{Gluc_SI_Transit_3} = k_T_Jej_2Cpt*\text{Gluc_SI3}$</td>
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<td>Flux of glucuronides from ileal lumen 1 to ileal lumen 2</td>
<td>$\text{Gluc_SI_Transit_4} = k_T_Ile_4Cpt*\text{Gluc_SI4}$</td>
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<td>Flux of glucuronides from ileal lumen 2 to ileal lumen 3</td>
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</tr>
<tr>
<td>Flux of glucuronides from ileal lumen 3 to ileal lumen 4</td>
<td>$\text{Gluc_SI_Transit_6} = k_T_Ile_4Cpt*\text{Gluc_SI6}$</td>
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</tr>
<tr>
<td>Flux of glucuronides from ileal lumen 4 to colon lumen 1</td>
<td>$\text{Gluc_SI_Transit_7} = k_T_Ile_4Cpt*\text{Gluc_SI7}$</td>
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<tr>
<td>Flux of glucuronides from colon lumen 1 to colon lumen 2</td>
<td>$\text{Gluc_Tran_Colon1} = k_T_Colon_5Cpt*\text{Gluc_Colon1}$</td>
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<tr>
<td>Flux of glucuronides from colon lumen 2 to colon lumen 3</td>
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<tr>
<td>Flux of glucuronides from colon lumen 3 to colon lumen 4</td>
<td>$\text{Gluc_Tran_Colon3} = k_T_Colon_5Cpt*\text{Gluc_Colon3}$</td>
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<tr>
<td>Flux of glucuronides from colon lumen 4 to colon lumen 5</td>
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<tr>
<td>Flux of glucuronides from colon lumen 5 to feces</td>
<td>$\text{Gluc_Tran_Colon5} = k_T_Colon_5Cpt*\text{Gluc_Colon5}$</td>
<td></td>
</tr>
<tr>
<td>Description</td>
<td>Formula</td>
<td>Description</td>
</tr>
<tr>
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</tr>
<tr>
<td>Hydrolysis_Colon_1</td>
<td>Gluc_k_hydrolysis*Gluc_Colon1</td>
<td>Flux of glucuronides to parent within colon lumen 1</td>
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<tr>
<td>Hydrolysis_Colon_2</td>
<td>Gluc_k_hydrolysis*Gluc_Colon2</td>
<td>Flux of glucuronides to parent within colon lumen 2</td>
</tr>
<tr>
<td>Hydrolysis_Colon_3</td>
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<td>Flux of glucuronides to parent within colon lumen 3</td>
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<td>Flux of glucuronides to parent within colon lumen 4</td>
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<tr>
<td>Hydrolysis_Colon_5</td>
<td>Gluc_k_hydrolysis*Gluc_Colon5</td>
<td>Flux of glucuronides to parent within colon lumen 5</td>
</tr>
<tr>
<td>Hydrolysis_Ileum3</td>
<td>Gluc_k_hydrolysis*Gluc_SI6</td>
<td>Flux of glucuronides to parent within ileal lumen 3</td>
</tr>
<tr>
<td>Hydrolysis_Ileum4</td>
<td>Gluc_k_hydrolysis*Gluc_SI7</td>
<td>Flux of glucuronides to parent within ileal lumen 4</td>
</tr>
<tr>
<td>QCHep</td>
<td>(k_h2C*Drug_Liver)<em>Liver-(k_C2h</em>Drug_Central)*Central</td>
<td>Flux of parent between liver and central compartments</td>
</tr>
<tr>
<td>QCP</td>
<td>(Drug_k12*Drug_Central)<em>Central-(Drug_k21</em>Drug_Peripheral)*Peripheral</td>
<td>Flux of parent between central and peripheral compartments</td>
</tr>
</tbody>
</table>
All differential equations used in the semi-physiologically based pharmacokinetic model for maribavir in cynomolgus monkeys. The fluxes on the right side of the differential equations are specified in Supplemental Table 1.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{d(Dose_{\text{IV}})}{dt} = -\text{Drug}_{\text{ka IV}} )</td>
<td>( \text{IV} ) dose rate</td>
</tr>
<tr>
<td>( \frac{d(Dose_{\text{PO}})}{dt} = -\text{Drug}_{\text{Stomach Transit}} )</td>
<td>( \text{PO} ) dose rate</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Central}})}{dt} = \frac{1}{\text{Central}^*}(-\text{QCP} - \text{CL}<em>R + \text{QCHep} - \text{Gluc}</em>\text{liver exc} + \text{Drug}_{\text{ka IV}}) )</td>
<td>Central compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Peripheral}})}{dt} = \frac{1}{\text{Peripheral}^*}(\text{QCP}) )</td>
<td>Peripheral compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Liver}})}{dt} = \frac{1}{\text{Liver}^*}(\text{Absorption}<em>1 + \text{Absorption}</em>\text{Colon1} + \text{Absorption}<em>2 + \text{Absorption}<em>3 + \text{Absorption}<em>4 + \text{Absorption}<em>5 - \text{Biliary secretion liver} - \text{QCHep} - \text{Gluc}</em>\text{from Liver} - \text{Drug}</em>\text{Liver Met1} + \text{Absorption}</em>\text{Colon2} + \text{Absorption}</em>\text{Colon3} + \text{Absorption}<em>\text{Colon4} + \text{Absorption}</em>\text{Colon5} + \text{Absorption}_6 + \text{Absorption}_7) )</td>
<td>Liver compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI1}})}{dt} = -\text{Absorption}<em>1 + \text{Drug}</em>{\text{Stomach Transit}} - \text{Drug}_\text{Transit SI 1} + \text{Biliary secretion liver} )</td>
<td>SI1 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI2}})}{dt} = \text{Drug}<em>\text{Transit SI 1} - \text{Drug}</em>\text{Transit SI 2} - \text{Absorption}_2 )</td>
<td>SI2 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI3}})}{dt} = \text{Drug}_\text{Transit SI 2} - \text{Absorption}<em>3 - \text{Drug}</em>\text{Transit SI 3} )</td>
<td>SI3 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI4}})}{dt} = \text{Drug}_\text{Transit SI 3} - \text{Absorption}<em>4 - \text{Drug}</em>\text{Transit SI 4} )</td>
<td>SI4 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI5}})}{dt} = \text{Drug}_\text{Transit SI 4} - \text{Absorption}<em>5 - \text{Drug}</em>\text{Transit SI 5} )</td>
<td>SI5 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI6}})}{dt} = \text{Drug}<em>\text{Transit SI 5} - \text{Drug}</em>\text{Transit SI 6} + \text{Hydrolysis Ileum3} - \text{Absorption}_6 )</td>
<td>SI6 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{SI7}})}{dt} = \text{Drug}_\text{Transit SI 6} + \text{Hydrolysis Ileum4} - \text{Absorption}<em>7 - \text{Drug}</em>\text{transit SI 7} )</td>
<td>SI7 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Colon1}})}{dt} = \text{Hydrolysis Colon 1} - \text{Absorption Colon1} - \text{Drug}<em>\text{Trans Colon1} + \text{Drug}</em>\text{transit SI 7} )</td>
<td>Colon1 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Colon2}})}{dt} = \text{Drug}<em>\text{Trans Colon1} + \text{Hydrolysis Colon 2} - \text{Drug}</em>\text{Trans Colon2} - \text{Absorption Colon2} )</td>
<td>Colon2 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Colon3}})}{dt} = \text{Drug}<em>\text{Trans Colon2} + \text{Hydrolysis Colon 3} - \text{Drug}</em>\text{Trans Colon3} )</td>
<td>Colon3 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Colon4}})}{dt} = \text{Hydrolysis Colon 4} + \text{Drug}<em>\text{Trans Colon3} - \text{Drug}</em>\text{Trans Colon4} )</td>
<td>Colon4 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Colon5}})}{dt} = \text{Hydrolysis Colon 5} + \text{Drug}<em>\text{Trans Colon4} - \text{Drug}</em>\text{Trans Colon5} )</td>
<td>Colon5 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Drug_{\text{Fecal}})}{dt} = \text{Drug}_\text{Trans Colon5} )</td>
<td>Fecal compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{\text{SI1}})}{dt} = -\text{Gluc}<em>\text{SI Transit 1} + \text{Gluc}</em>\text{from Liver} )</td>
<td>Gluc SI1 compartment dynamics</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{\text{SI2}})}{dt} = \text{Gluc}<em>\text{SI Transit 1} - \text{Gluc}</em>\text{SI Transit 2} )</td>
<td>Gluc SI2 compartment dynamics</td>
</tr>
<tr>
<td>Differential Equation</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{SI3})}{dt} )</td>
<td>( Gluc_{SI_Transit_2} - Gluc_{SI_Transit_3} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{SI4})}{dt} )</td>
<td>( Gluc_{SI_Transit_3} - Gluc_{SI_Transit_4} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{SI5})}{dt} )</td>
<td>( -Gluc_{SI_Transit_5} + Gluc_{SI_Transit_4} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{SI6})}{dt} )</td>
<td>( Gluc_{SI_Transit_5} - Gluc_{SI_Transit_6} - Hydrolysis_{Ileum3} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{SI7})}{dt} )</td>
<td>( Gluc_{SI_Transit_6} - Hydrolysis_{Ileum4} - Gluc_{SI_transit_7} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Colon1})}{dt} )</td>
<td>( -Hydrolysis_{Colon_1} - Gluc_{Tran_Colon1} + Gluc_{SI_transit_7} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Colon2})}{dt} )</td>
<td>( Gluc_{Tran_Colon1} - Hydrolysis_{Colon_2} - Gluc_{Tran_Colon2} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Colon3})}{dt} )</td>
<td>( Gluc_{Tran_Colon2} - Hydrolysis_{Colon_3} - Gluc_{Tran_Colon3} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Colon4})}{dt} )</td>
<td>( -Hydrolysis_{Colon_4} + Gluc_{Tran_Colon3} - Gluc_{Tran_Colon4} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Colon5})}{dt} )</td>
<td>( -Hydrolysis_{Colon_5} + Gluc_{Tran_Colon4} - Gluc_{Tran_Colon5} )</td>
</tr>
<tr>
<td>( \frac{d(Gluc_{Fecal})}{dt} )</td>
<td>( Gluc_{Tran_Colon5} )</td>
</tr>
<tr>
<td>( \frac{d(Amt_Drug_exc)}{dt} )</td>
<td>( CL_R )</td>
</tr>
<tr>
<td>( \frac{d(Amt_Drug_met1)}{dt} )</td>
<td>( Drug_Liver_Met1 )</td>
</tr>
<tr>
<td>( \frac{d(Amt_Gluc_exc)}{dt} )</td>
<td>( Gluc_liver_exc )</td>
</tr>
<tr>
<td>( \frac{d(Amt_Gluc_exc)}{dt} )</td>
<td>( Gluc_liver_exc )</td>
</tr>
</tbody>
</table>
SUPPLEMENTAL TABLE 3

Calculations of first-order absorption rate ($k_a$) in different segments of the intestine based on the surface area expansion factor (SAEF) and radii (Olivares-Morales et al., 2015).

<table>
<thead>
<tr>
<th>Intestinal segment</th>
<th>Radius (cm)</th>
<th>SAEF</th>
<th>Calculated first-order absorption rate ($k_a$) in monkey (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>1.75 in human, 0.4 in cynomolgus monkey</td>
<td>1</td>
<td>2.34</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2.37 in human</td>
<td>0.49</td>
<td>0.847</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.50 in human</td>
<td>0.58</td>
<td>1.58</td>
</tr>
<tr>
<td>Colon</td>
<td>2.42 in human</td>
<td>0.033</td>
<td>0.0558</td>
</tr>
</tbody>
</table>
**SUPPLEMENTAL TABLE 4**

Human intestinal effective permeability ($P_{eff}$) and apparent permeability ($P_{app}$) across the Caco-2 cell monolayer for reference drugs in historic studies and for maribavir. The rightmost column shows the difference of $\text{Ln}(P_{app})$ of propranolol and atenolol in previous studies to those measured in the current study with maribavir.

$\text{Ln}$, natural log.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Human intestinal $P_{eff}$ (Larregieu and Benet, 2013)</th>
<th>Caco-2 $P_{app}$ (Alsenz et al., 2003)</th>
<th>Caco-2 $P_{app}$ (Li et al., 2007)</th>
<th>Caco-2 $P_{app}$ in study with maribavir</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured value ($\times 10^{-4}$ cm/s)</td>
<td>$\text{Ln}$ (value)</td>
<td>Measured value ($\times 10^{-6}$ cm/s)</td>
<td>$\text{Ln}$ (value)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>2.91</td>
<td>1.07</td>
<td>47.2</td>
<td>3.85</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>1.34</td>
<td>0.293</td>
<td>31.8</td>
<td>3.46</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.20</td>
<td>$-1.61$</td>
<td>1.73</td>
<td>0.548</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>0.26</td>
<td>$-1.35$</td>
<td>0.59</td>
<td>$-0.528$</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>0.27</td>
<td>$-1.31$</td>
<td>0.67</td>
<td>$-0.400$</td>
</tr>
<tr>
<td>Maribavir</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SUPPLEMENTAL TABLE 5

All compartments, species, and parameters and their values used in the semi-physiologically based pharmacokinetic model for maribavir in cynomolgus monkeys. “L” denotes that the species were contained in the corresponding compartment above it. The SimBiology® diagram is depicted in Supplemental Fig. 1.

BDC, bile duct cannulated; CL, clearance.

<table>
<thead>
<tr>
<th>Compartment and species</th>
<th>Description</th>
<th>Value or unit</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Stomach lumen</td>
<td>0.1 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Dose_PO</td>
<td>Oral dose</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>Duodenal lumen</td>
<td>0.015 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI1</td>
<td>Amount of parent in duodenal lumen</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>L Gluc_SI1</td>
<td>Amount of maribavir glucuronides in duodenal lumen</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Jejunum_1</td>
<td>Jejunal lumen compartment 1</td>
<td>0.016 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI2</td>
<td>Amount of parent in jejunal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>L Gluc_SI2</td>
<td>Amount of maribavir glucuronides in jejunal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Jejunum_2</td>
<td>Jejunal lumen compartment 2</td>
<td>0.016 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI3</td>
<td>Amount of parent in jejunal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>L Gluc_SI3</td>
<td>Amount of maribavir glucuronides in jejunal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Ileum_1</td>
<td>Ileal lumen compartment 1</td>
<td>0.010 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI4</td>
<td>Amount of parent in ileal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>L Gluc_SI4</td>
<td>Amount of maribavir glucuronides in ileal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Ileum_2</td>
<td>Ileal lumen compartment 2</td>
<td>0.010 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI5</td>
<td>Amount of parent in ileal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>L Gluc_SI5</td>
<td>Amount of maribavir glucuronides in ileal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Ileum_3</td>
<td>Ileal lumen compartment 3</td>
<td>0.010 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>L Drug_SI6</td>
<td>Amount of parent in ileal lumen 3</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Compartment</td>
<td>Description</td>
<td>Volume</td>
<td>Source</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------------------------</td>
<td>--------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Ileum_4</td>
<td>Ileal lumen compartment 4</td>
<td>0.010 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Gluc_SI6</td>
<td>Amount of maribavir glucuronides in ileal lumen 3</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Drug_SI7</td>
<td>Amount of parent in ileal lumen 4</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_SI7</td>
<td>Amount of maribavir glucuronides in ileal lumen 4</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Colon_1</td>
<td>Colon lumen compartment 1</td>
<td>0.0292 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Colon1</td>
<td>Amount of parent in ileal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Colon1</td>
<td>Amount of maribavir glucuronides in ileal lumen 1</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Colon_2</td>
<td>Colon lumen compartment 2</td>
<td>0.0292 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Colon2</td>
<td>Amount of parent in ileal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Colon2</td>
<td>Amount of maribavir glucuronides in ileal lumen 2</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Colon_3</td>
<td>Colon lumen compartment 3</td>
<td>0.0292 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Colon3</td>
<td>Amount of parent in ileal lumen 3</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Colon3</td>
<td>Amount of maribavir glucuronides in ileal lumen 3</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Colon_4</td>
<td>Colon lumen compartment 4</td>
<td>0.0292 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Colon4</td>
<td>Amount of parent in ileal lumen 4</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Colon4</td>
<td>Amount of maribavir glucuronides in ileal lumen 4</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Colon_5</td>
<td>Colon lumen compartment 5</td>
<td>0.0292 L</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Colon5</td>
<td>Amount of parent in ileal lumen 5</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Colon5</td>
<td>Amount of maribavir glucuronides in ileal lumen 5</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>A generic fecal compartment</td>
<td>0.05 L</td>
<td></td>
</tr>
<tr>
<td>Drug_Fecal</td>
<td>Amount of parent in feces</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Gluc_Fecal</td>
<td>Amount of maribavir glucuronides in feces</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Liver compartment</td>
<td>0.027 L/kg</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>Drug_Liver</td>
<td>Drug concentration in the liver</td>
<td>in mg/L</td>
<td>Initial value set at 1E-9 mg/L</td>
</tr>
<tr>
<td>Central</td>
<td>Central compartment</td>
<td>in L</td>
<td>Value from fitting the BDC group data</td>
</tr>
<tr>
<td>Drug_Central</td>
<td>Drug concentration in the central compartment</td>
<td>in mg/L</td>
<td>Initial value set at 1E-9 mg/L</td>
</tr>
<tr>
<td>Dose_IV</td>
<td>Intravenous bolus dose</td>
<td>in mg</td>
<td></td>
</tr>
<tr>
<td>Parameter in SimBiology®</td>
<td>Description</td>
<td>Value</td>
<td>Notes</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>BW_Ref</td>
<td>Reference body weight</td>
<td>4.0 kg</td>
<td>Average body weight in the mass balance study</td>
</tr>
<tr>
<td>k_T_Stomach</td>
<td>Rate of transit from stomach to duodenum</td>
<td>2 h⁻¹</td>
<td>From fasted state, gastric emptying time of 30 min</td>
</tr>
<tr>
<td>SIRadius</td>
<td>Radius of the small intestine in cynomolgus monkey</td>
<td>0.4 cm</td>
<td>From Sugano et al., 2012</td>
</tr>
<tr>
<td>SITT</td>
<td>Small intestine transit time</td>
<td>2.7 h</td>
<td>From Ikegami et al., 2003</td>
</tr>
<tr>
<td>k_T_Duod</td>
<td>Duodenal lumen transit rate; defined as 1/(0.08*SITT), where 0.08 is the fractional length of duodenum within the small intestine</td>
<td>4.63 h⁻¹</td>
<td>Fractional length from Olivares-Morales et al., 2015</td>
</tr>
<tr>
<td>k_T_Jej_2Cpt</td>
<td>Jejunal lumen transit rate (two segments); defined as 2/(0.37*SITT), where 0.37 is the fractional length of jejunum within the small intestine</td>
<td>2.00 h⁻¹</td>
<td>Fractional length from Olivares-Morales et al., 2015</td>
</tr>
<tr>
<td>k_T_Ile_4Cpt</td>
<td>Ileal lumen transit rate (four segments); defined as 4/(0.55*SITT), where 0.55 is the fractional length of ileum within the small intestine</td>
<td>2.69 h⁻¹</td>
<td>Fractional length from Olivares-Morales et al., 2015</td>
</tr>
<tr>
<td>k_T_Colon</td>
<td>Colon transit rate</td>
<td>0.0422 h⁻¹</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>k_T_Colon_5Cpt</td>
<td>Colon transit rate between its five compartments</td>
<td>0.211 h⁻¹</td>
<td>From 5 * k_T_Colon</td>
</tr>
<tr>
<td>Qh</td>
<td>Hepatic blood flow in cynomolgus monkey</td>
<td>2.60 L/h/kg</td>
<td>From Peters et al., 2012</td>
</tr>
<tr>
<td>k_C2h and k_h2C</td>
<td>Transfer rates between central and liver compartments; defined as: k_C2h = Qh / Central; k_h2C = Qh / Liver</td>
<td>in h⁻¹</td>
<td></td>
</tr>
<tr>
<td>Drug_Peff</td>
<td>Effective permeability in jejunum</td>
<td>1.30 × 10⁻⁴ cm/s</td>
<td>Calculated from Caco-2 cell data; see Supplemental Methods</td>
</tr>
<tr>
<td>Drug</td>
<td>Description</td>
<td>Value</td>
<td>Notes</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Drug_ka_Duodenum</td>
<td>First-order absorption rate in duodenum</td>
<td>0.847 h⁻¹</td>
<td>Calculations in Supplemental Methods</td>
</tr>
<tr>
<td>Drug_ka_Jejunum</td>
<td>First-order absorption rate in jejunum compartments 1 and 2</td>
<td>2.34 h⁻¹</td>
<td>Calculations in Supplemental Methods</td>
</tr>
<tr>
<td>Drug_ka_Ileum</td>
<td>First-order absorption rate in ileum compartments 1 through 4</td>
<td>1.58 h⁻¹</td>
<td>Calculations in Supplemental Methods</td>
</tr>
<tr>
<td>Drug_ka_Colon</td>
<td>First-order absorption rate in colon compartments 1 through 5</td>
<td>0.0558 h⁻¹</td>
<td>Calculations in Supplemental Methods</td>
</tr>
<tr>
<td>Drug_fm_gluc</td>
<td>Fraction metabolized by direct glucuronidation pathway</td>
<td>0.728 or 0.853</td>
<td>0.728 from in vivo data in BDC group (main text Table 5); 0.853 from in vitro hepatocyte data</td>
</tr>
<tr>
<td>Gluc_k_hydrolysis</td>
<td>Rate of hydrolysis of maribavir glucuronides</td>
<td>3.1 h⁻¹</td>
<td>See Supplemental Methods</td>
</tr>
<tr>
<td>Drug_CL_Renal</td>
<td>Renal clearance of parent from central compartment</td>
<td>0.0128 L/h</td>
<td>From BDC mean systemic CL × %dose in urine: 5.72 L/h × 0.224% (main text Tables 3 and 4)</td>
</tr>
<tr>
<td>Drug_ke_renal</td>
<td>Renal elimination rate of parent; defined as: Drug_CL_Renal / Central</td>
<td>in h⁻¹</td>
<td>From BDC mean systemic CL × %dose in bile: 5.72 L/h × 1.26% (main text Tables 3 and 4)</td>
</tr>
<tr>
<td>Drug_CL_Biliary</td>
<td>Biliary clearance of parent from central compartment</td>
<td>0.0719 L/h</td>
<td>Drug_CL_Biliary was derived from central concentrations. A reverse well-stirred model was necessary to empirically convert it to a liver-based clearance</td>
</tr>
<tr>
<td>Drug_Liver_CL_met</td>
<td>Metabolic clearance from the liver compartment; defined as: Drug_Liver_CL-Qh*Drug_CL_Biliary / (Qh-Drug_CL_Biliary)</td>
<td>in L/h</td>
<td>Drug_CL_Biliary was derived from central concentrations. A reverse well-stirred model was necessary to empirically convert it to a liver-based clearance</td>
</tr>
<tr>
<td>Drug_Liver_ke_gluc</td>
<td>Rate of maribavir glucuronidation and excretion to duodenum; defined as: Drug_Liver_CL_met * Drug_fm_gluc / Liver</td>
<td>in h⁻¹</td>
<td></td>
</tr>
<tr>
<td>Drug_Liver_ke_met1</td>
<td>Rate of non-glucuronidation metabolic elimination of parent; defined as: Drug_Liver_CL_met * (1-Drug_fm_gluc) / Liver</td>
<td>in h⁻¹</td>
<td></td>
</tr>
<tr>
<td>Drug_k12 and Drug_k21</td>
<td>Rate of transfer between central and peripheral compartments; defined as: Drug_k12 = Drug_Q12 / Central</td>
<td>in h⁻¹</td>
<td></td>
</tr>
</tbody>
</table>
Drug\_k21 = Drug\_Q12 / Peripheral

<table>
<thead>
<tr>
<th>Parameter in SimBiology®</th>
<th>Description</th>
<th>Value</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug_Q12</td>
<td>Central to peripheral transfer</td>
<td>0.660 L/h</td>
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<tr>
<td>Drug_Liver_CL</td>
<td>Drug clearance from the liver compartment</td>
<td>15.7 L/h</td>
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<tr>
<td>Drug_Vc_Ref</td>
<td>Volume of the central compartment</td>
<td>6.32 L</td>
<td></td>
</tr>
<tr>
<td>Drug_Vp_Ref</td>
<td>Volume of peripheral compartment</td>
<td>2.79 L</td>
<td></td>
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</tbody>
</table>
SUPPLEMENTAL TABLE 6

Tentative structures, characteristics, and matrices in which metabolites of maribavir were identified after a single intravenous bolus administration to male cynomolgus monkeys. Note that there is no sufficient information on differentiating the N- from the O-glucuronides among M1/7/16/17, nor to clearly locate the O-glucuronidation site among M10/11/12.

\([\text{M+H}^+]\), molecular weight of protonated compound with \(^{35}\text{Cl}\) isotope; Gluc, glucuronide; m/z, mass over net charge.

<table>
<thead>
<tr>
<th>Metabolite designation</th>
<th>([\text{M+H}^+])</th>
<th>Characteristic product ions (m/z)</th>
<th>Proposed metabolite structure</th>
<th>Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>M15</td>
<td>461</td>
<td>329, 240, 198, 85</td>
<td><img src="image" alt="Proposed metabolite structure for M15" /></td>
<td>Bile</td>
</tr>
<tr>
<td>M1</td>
<td>510</td>
<td>202, 167, 115, 85</td>
<td><img src="image" alt="Proposed metabolite structure for M1" /></td>
<td>Plasma, Urine</td>
</tr>
<tr>
<td>M7</td>
<td>510</td>
<td>202, 167, 115, 85</td>
<td><img src="image" alt="Proposed metabolite structure for M7" /></td>
<td>Plasma, Urine, Bile</td>
</tr>
<tr>
<td>M16</td>
<td>510</td>
<td>202, 167, 85</td>
<td><img src="image" alt="Proposed metabolite structure for M16" /></td>
<td>Urine, Bile</td>
</tr>
<tr>
<td>M17</td>
<td>510</td>
<td>202, 167, 115, 85</td>
<td><img src="image" alt="Proposed metabolite structure for M17" /></td>
<td>Plasma, Urine, Bile</td>
</tr>
<tr>
<td>Compound</td>
<td>MW</td>
<td>Retention Times (min)</td>
<td>Metabolites</td>
<td>Excretion Routes</td>
</tr>
<tr>
<td>----------</td>
<td>----</td>
<td>----------------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>M2</td>
<td>420</td>
<td>244, 202, 167</td>
<td><img src="image" alt="Structure" /></td>
<td>Plasma, Urine, Bile, Feces</td>
</tr>
<tr>
<td>M10</td>
<td>552</td>
<td>244, 202, 167, 115, 85</td>
<td><img src="image" alt="Structure" /></td>
<td>Urine</td>
</tr>
<tr>
<td>M4</td>
<td>334</td>
<td>202, 167, 133, 115, 85</td>
<td><img src="image" alt="Structure" /></td>
<td>Plasma, Urine, Feces</td>
</tr>
<tr>
<td>M5</td>
<td>392</td>
<td>260, 202</td>
<td><img src="image" alt="Structure" /></td>
<td>Urine, Feces</td>
</tr>
<tr>
<td>M11</td>
<td>552</td>
<td>244, 202, 167, 115, 85</td>
<td><img src="image" alt="Structure" /></td>
<td>Plasma, Urine, Bile</td>
</tr>
<tr>
<td>M12</td>
<td>552</td>
<td>244, 202, 167, 115, 85</td>
<td><img src="image" alt="Structure" /></td>
<td>Plasma, Urine, Bile</td>
</tr>
<tr>
<td>Maribavir (SHP620)</td>
<td>376</td>
<td>244, 202, 167, 133, 115, 85</td>
<td><img src="image" alt="Structure" /></td>
<td>Plasma, Urine, Bile, Feces</td>
</tr>
</tbody>
</table>
SUPPLEMENTAL TABLE 7

(A) Percentage of sample radioactivity as $^{14}$C-maribavir or metabolites of $^{14}$C-maribavir in pooled plasma samples after a single intravenous dose of $^{14}$C-maribavir to male intact monkeys (Group 1 [$n = 3$], 13 mg/kg). (B) Percentage of sample radioactivity as $^{14}$C-maribavir or metabolites of $^{14}$C-maribavir in pooled plasma samples after a single intravenous dose of $^{14}$C-maribavir to male bile duct-cannulated monkeys (Group 2 [$n = 3$], 13 mg/kg). ND, peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height). Note that the samples were not pooled based on the Hamilton method (Hamilton et al., 1981) and that individuals may exhibit slightly different circulating parent and metabolite profiles.

### A

<table>
<thead>
<tr>
<th>Metabolite designation</th>
<th>Collection time (Hours)</th>
<th>Percent of radioactivity injected (% of run)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.083</td>
<td>0.25</td>
</tr>
<tr>
<td>M1</td>
<td>90.4</td>
<td>80.3</td>
</tr>
<tr>
<td>M7</td>
<td>2.60</td>
<td>3.10</td>
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<tr>
<td>M17/M2</td>
<td>4.79</td>
<td>6.36</td>
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<tr>
<td>M4</td>
<td>1.78</td>
<td>4.96</td>
</tr>
<tr>
<td>M11</td>
<td>1.20</td>
<td>2.90</td>
</tr>
<tr>
<td>M12</td>
<td>3.07</td>
<td>4.45</td>
</tr>
<tr>
<td>Parent</td>
<td>99.6</td>
<td>98.9</td>
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<tr>
<td>TOTAL</td>
<td>99.6</td>
<td>98.9</td>
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</table>

### B

<table>
<thead>
<tr>
<th>Metabolite designation</th>
<th>Collection time (Hours)</th>
<th>Percent of radioactivity injected (% of run)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.083</td>
<td>0.25</td>
</tr>
<tr>
<td>M1</td>
<td>88.9</td>
<td>81.0</td>
</tr>
<tr>
<td>M7</td>
<td>3.07</td>
<td>3.87</td>
</tr>
<tr>
<td>M17/M2</td>
<td>1.20</td>
<td>2.90</td>
</tr>
<tr>
<td>M4</td>
<td>2.40</td>
<td>4.45</td>
</tr>
<tr>
<td>M11</td>
<td>88.9</td>
<td>81.0</td>
</tr>
<tr>
<td>M12</td>
<td>3.07</td>
<td>3.87</td>
</tr>
<tr>
<td>Parent</td>
<td>98.7</td>
<td>99.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>98.7</td>
<td>99.6</td>
</tr>
</tbody>
</table>
SUPPLEMENTAL TABLE 8

Segmental absorption percentages of maribavir in the cynomolgus monkey gastrointestinal tract, followed by intravenous bolus or oral dosing (Table 5, main text), as predicted by the semi-PBPK model. For i.v. dosing, the reabsorption does not occur in duodenum, jejunum, or upper ileum due to a lack of GUS-expressing bacteria under normal conditions (grayed out cells). For oral dosing, the overall fraction absorbed ($F_a$) is 67% at $f_m$(Gluc) = 0.728.

<table>
<thead>
<tr>
<th>GI segment in the model</th>
<th>5 mg/kg intravenous bolus</th>
<th>10 mg/kg oral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (h*mg/L) contributed from segment</td>
<td>Percent contribution to the reabsorbed amount</td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jejunum 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileum 3</td>
<td>0.21</td>
<td>11%</td>
</tr>
<tr>
<td>Ileum 4</td>
<td>0.34</td>
<td>18%</td>
</tr>
<tr>
<td>Colon 1</td>
<td>0.26</td>
<td>14%</td>
</tr>
<tr>
<td>Colon 2</td>
<td>0.27</td>
<td>14%</td>
</tr>
<tr>
<td>Colon 3</td>
<td>0.28</td>
<td>15%</td>
</tr>
<tr>
<td>Colon 4</td>
<td>0.26</td>
<td>14%</td>
</tr>
<tr>
<td>Colon 5</td>
<td>0.25</td>
<td>13%</td>
</tr>
</tbody>
</table>
Supplemental Fig. 1. SimBiology® diagram for the semi-physiologically based pharmacokinetic model for maribavir in monkeys. Circles, empty large rectangles, and filled small rectangles denote mass transfers, compartments, and species, respectively, and are detailed in Supplemental Table 1.
Supplemental Fig. 2. Linear regressions of natural logs (Ln) of human intestinal permeability ($P_{\text{eff}}$, in $10^{-4}$ cm/s; data in Supplemental Table 4 and from Larregieu and Benet, 2013) versus Ln of apparent permeability ($P_{\text{app}}$, in $10^{-6}$ cm/s) across cultured Caco-2 cell monolayers. (A) Caco-2 $P_{\text{app}}$ data from Alsenz et al., 2003; (B) Caco-2 $P_{\text{app}}$ data from Li et al., 2007. Equations show the results from linear regression.

Aten, atenolol; Cim, cimetidine; Meto, metoprolol; $P_{\text{app}}$, apparent permeability; $P_{\text{eff}}$, effective permeability; Prop, propranolol; Ran, ranitidine.
Supplemental Fig. 3. Diagnostic graphs after fitting the semi-physiologically based pharmacokinetic model to observed plasma concentration versus time data in the bile duct-cannulated group of cynomolgus monkeys. (A) Observation (mg/L, y axis) versus predictions (mg/L, x axis). Blue line denotes unity; inset shows the same graph with x and y axes in log scale. (B) Weighted residuals (mg/L, y axis) versus predictions (mg/L, x axis). Blue line denotes y = 0.
Supplemental Fig. 4. Total radioactivity in plasma as determined by LSC (diamonds) and concentration of maribavir in plasma determined by LC-MS/MS (hexagons) versus time profile after a single i.v. bolus administration of 13 mg/kg $^{14}$C-maribavir to intact or BDC animals. Note that the time scale (x axis) for intact and BDC animals are different. Missing samples and BLQ data are not plotted. BDC, bile duct-cannulated; BLQ, below the limit of quantitation; i.v., intravenous; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LSC, liquid scintillation counters.
Structure and proposed fragmentation pattern

Supplemental Fig. 5. Product ion (m/z 376) mass spectrum of maribavir from analysis of a standard solution of maribavir.
Supplemental Fig. 6. Product ion (m/z 376) mass spectrum of maribavir from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg)
Proposed structure and fragmentation pattern

Supplemental Fig. 7. Product ion (m/z 461) mass spectrum of metabolite M15 from analysis of a 0- to 8-hour pooled bile sample after a single intravenous dose of 14C-maribavir to male bile-duct cannulated monkeys (Group 2, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 8. Product ion (m/z 510) mass spectrum of metabolite M1 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 9. Product ion (m/z 510) mass spectrum of metabolite M7 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 10. Product ion (m/z 510) mass spectrum of metabolite M16 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 11. Product ion (m/z 510) mass spectrum of metabolite M17 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

![Proposed structure and fragmentation pattern](image)

**Supplemental Fig. 12.** Product ion (m/z 420) mass spectrum of metabolite M2 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 13. Product ion (m/z 552) mass spectrum of metabolite M10 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 14. Product ion (m/z 334) mass spectrum of metabolite M4 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

Supplemental Fig. 15. Product ion (m/z 392) mass spectrum of metabolite M5 from analysis of a 0- to 120-hour pooled feces sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg)
Proposed structure and fragmentation pattern

Supplemental Fig. 16. Product ion ($m/z$ 552) mass spectrum of metabolite M11 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Proposed structure and fragmentation pattern

[Diagram showing the proposed structure and fragmentation pattern of metabolite M12]

Supplemental Fig. 17. Product ion (m/z 552) mass spectrum of metabolite M12 from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Supplemental Fig. 18. Extracted ion chromatogram from analysis of a standard solution of maribavir (SHP620).
Supplemental Fig. 19. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0.25-hour pooled plasma sample after a single intravenous dose of $^{14}$C-maribavir to intact male monkeys (Group 1, 13 mg/kg)
Supplemental Fig. 20. Reconstructed ion chromatogram and radiochromatogram from analysis of a 2-hour pooled plasma sample after a single intravenous dose of $^{14}$C-maribavir to male bile-duct cannulated monkeys (Group 2, 13 mg/kg).
Supplemental Fig. 21. Reconstructed ion chromatogram and radiochromatogram from analysis of a 24-hour pooled plasma sample after a single intravenous dose of $^{14}$C-maribavir to intact male monkeys (Group 1, 13 mg/kg).
Supplemental Fig. 22. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0- to 48-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to intact male monkeys (Group 1, 13 mg/kg).
Supplemental Fig. 23. Reconstructed ion chromatogram and radiochromatogram from analysis of a 120- to 144-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to intact male monkeys (Group 1, 13 mg/kg).
Supplemental Fig. 24. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0- to 24-hour pooled urine sample after a single intravenous dose of $^{14}$C-maribavir to male bile-duct cannulated monkeys (Group 2, 13 mg/kg).
Supplemental Fig. 25. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0- to 8-hour pooled bile sample after a single intravenous dose of $^{14}$C-maribavir to male bile-duct cannulated monkeys (Group 2, 13 mg/kg).
Supplemental Fig. 26. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0- to 120-hour pooled feces sample after a single intravenous dose of $^{14}$C-maribavir to male monkeys (Group 1, 13 mg/kg).
Supplemental Fig. 27. Reconstructed ion chromatogram and radiochromatogram from analysis of a 0- to 24-hour pooled feces sample after a single intravenous dose of $^{14}$C-maribavir to male bile-duct cannulated monkeys (Group 2, 13 mg/kg).
A

Observed vs. Predicted ($f_m$(Gluc) 0.728)

B

Observed vs. Predicted ($f_m$(Gluc) 0.853)
Supplemental Fig. 28. Observed versus PBPK model-predicted concentration plots for plasma concentration data in 13 mg/kg $^{14}$C-maribavir and 5 mg/kg maribavir intravenous bolus administration in intact cynomolgus monkeys using (A) $f_{m}(\text{Gluc}) = 0.728$ or (B) $f_{m}(\text{Gluc}) = 0.853$ for the prediction. Each plot contains the same mean observed concentration data (LC-MS/MS) from both dose groups (circles) and the y error bars denote standard deviation. The BLQ concentrations at 120- and 144-hour samples in the two 13 mg/kg $^{14}$C-maribavir-dosed animals were treated as zeros. The solid line represents unity and the two dotted lines represent 0.5x and 2x Observed / Predicted ratios.
Supplemental Fig. 29. Additional sensitivity analyses for effects of parameters in the semi-physiologically based pharmacokinetic model on simulated maribavir plasma pharmacokinetic profile after a single 10 mg/kg oral dose. Parameters analyzed included (A) intestinal $P_{eff}$, (B) Drug_Liver_CL, (C) SITT, (D) rate of hydrolysis of maribavir glucuronides (Gluc_k_hydrolysis), and (E) intercompartmental drug clearance between the central and peripheral compartments (Drug_Q12).

SITT, small intestine transit time.
Supplemental References


