Metabolism and Disposition of the Hepatitis C Protease Inhibitor Paritaprevir in Humans

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Received April 28, 2016; accepted May 11, 2016

ABSTRACT

Paritaprevir (also known as ABT-450), a potent NS3-4A serine protease inhibitor [identified by AbbVie (North Chicago, IL) and Enanta Pharmaceuticals (Watertown, MA) of the hepatitis C virus (HCV), has been developed in combination with ombitasvir and dasabuvir in a three-direct-acting antiviral agent (DAA) oral regimen for the treatment of patients infected with HCV genotype 1. This article describes the mass balance, metabolism, and disposition of paritaprevir in humans. After the administration of a single 200-mg oral dose of [14C]paritaprevir coadministered with 100 mg of ritonavir to four male healthy volunteers, the mean total percentage of the administered radioactive dose recovered was 96.5%, with recovery in individual subjects ranging from 96.0% to 96.9%. Radioactivity derived from [14C]paritaprevir was primarily eliminated in feces (87.8% of the dose). Radioactivity recovered in urine accounted for 8.8% of the dose. The biotransformation of paritaprevir in humans involves: 1) P450-mediated oxidation on the olefinic linker, the phenanthridine group, the methylpyrazinyl group, or combinations thereof; and 2) amide hydrolysis at the acyl cyclopropane-sulfonamide moiety and the pyrazine-2-carboxamide moiety. Paritaprevir was the major component in plasma [80.1% of total radioactivity in plasma, AUC from time 0 to 12 hours (AUC0–12h)] pool]. Five minor metabolites were identified in plasma, including the metabolites M2, M29, M3, M13, and M6; none of the metabolites accounted for greater than 10% of the total radioactivity. Paritaprevir was primarily eliminated through the biliary-fecal route followed by microflora-mediated sulfonamide hydrolysis to M29 as a major component in feces (approximately 60% of dose). In summary, the biotransformation and clearance pathways of paritaprevir were characterized, and the structures of metabolites in circulation and excreta were elucidated.

Introduction

Hepatitis C virus (HCV) is a virus that is a common cause of viral hepatitis. Untreated HCV can lead to chronic liver disease, including cirrhosis, organ failure, and liver cancer. An estimated 150–170 million people worldwide are chronically infected with HCV and have an increased risk of the development of liver cirrhosis or liver cancer (Lavanchy, 2011; Mohd Hanafiah et al., 2013). Genotype 1 is the most prevalent genotype in the United States, Europe, and Japan, and globally accounts for approximately 60% of HCV infections (Zein, 2000).

HCV is an enveloped positive-strand RNA virus that replicates primarily in the cytoplasm of hepatocytes. Viral NS3-4A serine protease is a noncovalent heterodimer consisting of a catalytic subunit [the N-terminal one-third of nonstructural (NS) 3 protein] and an activating cofactor (NS4A protein), which is responsible for cleavage of the HCV polyprotein at four sites. HCV NS3-4A protease is essential for the viral replication process (Lin, 2006; Moradpour et al., 2007) and is a validated drug target (McHutchison et al., 2009; Poordad et al., 2011). Paritaprevir is a potent macrocyclic noncovalent peptidomimetic inhibitor [identified by AbbVie (North Chicago, IL) and Enanta Pharmaceuticals (Watertown, MA)] of HCV NS3/4A protease, with 50% effective concentration values of 1.0, 0.21, 5.3, 19, 0.09, and 0.69 nM against stable HCV replicons with NS3 protease from genotypes 1a, 1b, 2a, 3a, 4a, and 6a, respectively (Pilot-Matias et al., 2015). Paritaprevir with ritonavir demonstrated robust in vivo responses with mean maximum decreases in HCV RNA from 3.89 to 4.11 log10 IU/ml after 3-day monotherapy in treatment-naive HCV genotype 1–infected subjects among three dose groups (Lawitz et al., 2010). Paritaprevir with ritonavir has been used in combination with the NSSA inhibitor ombitasvir (formerly known as ABT-267), the non-nucleoside NS5B polymerase inhibitor dasabuvir (formerly known as ABT-333) for treatment with interferon-free DAAs of HCV infection with or without ribavirin (Feld et al., 2014; Kowdley et al., 2014; Zeuzem et al., 2014). Treatment with paritaprevir/ombitasvir, and dasabuvir with ribavirin showed higher rates of sustained virologic response among

ABBREVIATIONS: 1-ABT, 1-aminobenzotriazole; ACN, acetonitrile; AUC, area under the curve; AUC0–12h, area under the curve for concentration at time 0 to the last dose; AUC0–12h, area under the curve from time 0 to 12 hours; CID, collisionally induced dissociation; Cmax, maximum plasma concentration; DAA, direct-acting antiviral agent; FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; HCV, hepatitis C virus; HPLC, high-performance liquid chromatography; IS, internal standard; LC, liquid chromatography; LSC, liquid scintillation counting; MS, mass spectrometry; MS2, tandem mass spectrometry; MS3, multistage mass spectrometry; NS, nonstructural protein; P450, cytochrome P450; SIF, simulated intestinal fluid; SPE, solid phase extraction; t1/2, half-life; Tmax, time at which maximum plasma concentration was achieved.

†This article has supplemental material available at dmd.aspetjournals.org.

http://dx.doi.org/10.1124/dmd.115.067488

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http://dx.doi.org/10.1124/dmd.115.067488

Drugs Metabolism & Disposition, Vol. 44, 1164–1173, August 2016

1521-009X/44/8/1164–1173$25.00 http://dx.doi.org/10.1124/dmd.115.067488

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Metabolism and Disposition of [14C]Paritaprevir in Humans

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Materials and Methods

Drugs and Reagents

Paritaprevir (2R,6S,12Z,13aS,14aR,16aS)-N-(cyclopropylsulfonyl)-6-[[5-methylpyrazin-2-yl(carbonyl)amino]-5,16-dioxo-2-phenanthridin-6-yl oxy]-1,2,3,6,7,8,9,10,11,13a,14,15,16a-tetradecahydrocyclopenta[e]pyrrolo[1,2-a][1,4]diazacyclopentadecine-14a(5H) carboxamidehydrate, [14C]paritaprevir, and metabolite M2 reference material were supplied by the Process Chemistry Department at AbbVie. The chemical structure of paritaprevir is shown in Fig. 1 (where * denotes the [14C] label position). The radiochemical synthesis of [14C]paritaprevir was conducted in three steps using [14C]potassium cyanide as the starting material. Radiochemical purity was >99% after HPLC purification. 5-Methylpyrazine 2-carboxylic acid (M13) was obtained from Sigma-Aldrich (St. Louis, MO). All these reference standards were used as HPLC and mass spectrometry (MS) standards for structure confirmation.

Clinical Study

The clinical study was conducted at Covance Laboratories Inc. (Madison, WI), in conjunction with the Covance Clinical Research Unit (Madison, WI). In this open-label study, a total of four adult male subjects (N = 4) who were in general good health were selected to participate in the study according to the selection criteria. On the morning of study day 1, subjects received a single oral dose of [14C]paritaprevir and ritonavir under nonfasting conditions. The study drug, paritaprevir (200 mg active, 100 μCi [14C]) and ritonavir 100 mg was administered as four (4 × 50 mg) hard gelatin capsules of paritaprevir and one capsule of ritonavir (100 mg). The total amount of liquid taken was approximately 240 ml, 30 minutes after starting a standardized breakfast. Subjects were confined to the study site for a minimum of 120 hours postdose, or up to a maximum of 312 hours postdose.

Blood samples were collected by venipuncture into potassium EDTA vacutainer collection tubes at the following times: 0 hour (predose), and 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, and 192 hours postdose. Plasma was separated via centrifugation and stored at −70°C.

Urine samples were collected over the following intervals: 0–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168, and 168–192 hours postdose. All feces collected during a collection interval were kept frozen at −70°C.

Total Radioactivity Measurement by Liquid Scintillation Counting

All sample combustions were conducted using a Model 307 Sample Oxidizer (Packard Instrument Company, Inc., Meriden, CT), and the resulting 14CO2 was trapped in a mixture of Perma Fluor and Carbo Sorb (Perkin Elmer, Waltham, MA). The oxidation efficiency of sample combustion was evaluated daily by analyzing a commercial radiolabeled standard both directly in scintillation cocktail and by oxidation. Acceptance criteria were defined as combustion recoveries of 95–105%. Ultima Gold XR Scintillation Cocktail (Perkin Elmer) was used for samples analyzed directly. All samples were analyzed for radioactivity in Model 2900TR Liquid Scintillation Counters (Packard Instrument Company, Inc.) for at least 5 minutes or 100,000 counts. Each sample was homogenized or mixed with scintillation cocktail before radioanalysis.

All samples were analyzed in duplicate if sample size allowed. If results from sample replicates (calculated as 14C dpm/g sample) differed by more than 10% from the mean value and sample aliquots had radioactivity greater than 200 dpm, the sample was rehomogenized and reanalyzed.

Blood samples were mixed, and duplicate weighed aliquots (approximately 0.2 g) were combusted and analyzed by liquid scintillation counting (LSC). The representative lower limit of quantitation for blood was 92.1-ng equivalents/g. Plasma samples were mixed and duplicate weighed aliquots (approximately 0.2 g) were analyzed directly by LSC. The representative lower limit of quantitation for plasma was 71.7 ng equivalents/g. The urine samples were mixed and duplicate weighed aliquots (approximately 0.2 g) were analyzed directly by LSC. The representative lower limit of quantitation for urine was 71.7 ng equivalents/g. Fecal samples were combined across subjects at 24-hour intervals, and the weight of each combined sample was recorded. A weighed amount of water was added, and the sample was mixed. The sample was removed from the freezer and homogenized, or immediately homogenized using a probe-type homogenizer. Duplicate weighed aliquots (approximately 0.2 g) were combusted and analyzed by LSC.

Sample Preparation for Metabolite Profiling

Plasma samples were thawed at the room temperature and pooled across subjects at selected time points or by using the Hamilton method (also known as the time point–weighted AUC pool) (Hamilton et al., 1981) for each subject. Plasma samples were processed using a two-step extraction method. In brief, pooled plasma was extracted with a 2-fold volume of acetonitrile (ACN)/methanol (3:1, v/v), followed by sonication and vortexing. The quenched sample was centrifuged at 3000 rpm (2465g) for 15 minutes at 4°C. After collecting the supernatant, the protein pellets were extracted using a 3-fold volume of ACN/methanol (3:1, v/v) with vortexing and sonication. After centrifugation at 3000 rpm (2465g) for 15 minutes at 4°C, the supernatant was collected and combined with the previous extract. Combined supernatants from the extracts were completely evaporated under a nitrogen stream at room temperature. The residues were dissolved in 5 ml of ACN/methanol (3:1, v/v) for solid phase extraction (SPE) using the Agilent MEGA BE-C8 Bond Elut (part #12525002; Agilent Technologies, Santa Clara, CA). The cartridge was conditioned using 2 × 5 ml ACN/methanol (3:1, v/v) and 2 × 5 ml 0.1% formic acid in water. The
cartridge was prefilled with 4.5 ml of deionized water with the stopcock closed and 0.5 ml of the extracted sample was loaded, mixing well before opening the stopcock to initiate the flow through the cartridge. Once the sample was loaded, the process was repeated until all the extracted sample was loaded to the column. The cartridge was washed with 5 ml of deionized water, and the sample was eluted with 3 × 5 ml ACN/methanol (3:1, v/v). The eluent was evaporated to dryness under a nitrogen stream at room temperature, and the residue was reconstituted in 75 μl of ACN/methanol (3:1, v/v), diluted with 150 μl of water before HPLC-MS-radiochemical detection analysis. An aliquot of the reconstituted sample was subjected to LSC counting to determine the total radioactivity recovery. The mean radioactivity recovery in the processed plasma samples was about 83%.

Pooled urine was extracted using SPE. In brief, an SPE cartridge (Phenomenex Strata X-AW 33 μ Polymeric Weak Anion, 1 g/12 ml part #8B-S038-HDG; Phenomenex, Torrance, CA) was conditioned with 15 ml of methanol and 15 ml of deionized water. Aliquots of pooled urine were loaded to the preconditioned column, followed by washing with 10 ml of 10 mM ammonium acetate buffer solution. The elution was achieved by using 4 × 5 ml 0.1% formic acid in ACN/methanol (3:1, v/v). The eluent was dried down under the nitrogen stream at room temperature. The residue was reconstituted in initial mobile phase for HPLC-MS-radioactivity detection analysis. The overall extraction recovery was about 88%.

Pooled fecal samples were processed using multiple solvent extractions with ACN/methanol (3:1, v/v) using a 1.3 sample/solvent ratio, followed by centrifugation at 4000 rpm (3220g) for 20 minutes at 4°C. The extracted sample was stopped when either 80% of the radioactivity had been recovered or until less than 2% of the radioactivity was extracted. Aliquots of extracted samples were subjected to LSC counting for total radioactivity. The extract was dried down under the nitrogen stream at room temperature, and the final residues were reconstituted in initial mobile phase for liquid chromatography (LC)-MS-radiodetection analysis. An aliquot of the reconstituted solution was subject to LSC radio counting for extraction recovery calculation. The overall extraction recovery for fecal sample was about 92%.

Method for Metabolite Profiles and Identification

HPLC separation of paritaprevir and metabolites was conducted using a Thermo Accela UHPLC system (Thermo Fisher, San Jose, CA), which consisted of an UHPLC autosampler, a 1250 Series binary pump, and an Accela PDA detector. Separation was achieved at room temperature on a Phenomenex Synergy Polar-RP 4 μm, 80 A, 4.6 × 150 mm HPLC column. The HPLC mobile phase consisted of 10 mM ammonium formate (adjusted to pH 3.1 with formic acid) (solvent A) and 100% ACN (solvent B), and the flow rate was maintained at 1.0 ml/min. The gradient was as follows: 0–2 minutes, 20% solvent B; 2–60 minutes, 20–73% solvent B; 63–63.5 minutes, 95–20% solvent B; 63.5–68 minutes, 20% solvent B. The UHPLC system was interfaced with a Thermo Fisher Orbitrap Discovery Mass Spectrometer (ThermoFisher Scientific Waltham, MA). The MS analyses were conducted using electrospray ionization operated in positive ionization mode. The MS settings were as follows: electrospray ionization voltage, 4.0 kV; capillary temperature, 300°C; capillary voltage, 2 V; and tube lens, 105 V. The sheath gas was set to 55.0 arbitrary units, and auxiliary gas to 10 arbitrary units. The unchanged parent drug and its metabolites were detected using data-dependent, multiple-stage mass analysis with mass isolation of 2 Da, and normalized collision energy of 35% for both tandem MS (MS²) and multistage MS (MS³). The mass resolution was set for 30,000 for full scan and 7500 for MS² and MS³ scans. Accurate mass measurement was obtained using daily external calibration. Data acquisition and processing were carried out using Xcalibur 2.2 (ThermoFisher Scientific).

Radio labeled components in plasma, urine, or feces samples were detected by a PerkinElmer TopCount 96 Deep Well Luma Plate (PerkinElmer, Waltham, MA). The HPLC eluent was split postcolumn between the mass spectrometer and Agilent 1100 Fraction Collector (Agilent Technologies, Wilmington, DE) at a ratio of 20:80. The Agilent 1100 Fraction Collector was set at a 0.3-minute interval per well collection. Radioactivity counting was conducted using the PerkinElmer TopCount NXT System.

Quantitation of Metabolite M2 in Human Plasma

A single liquid-liquid extraction with a mixture of ethyl acetate and hexane was used to extract the compounds of interest from plasma. An aliquot of plasma (200 μl sample or spiked standard) was combined with 50 μl of internal standard (IS) [(1S,3S,4S)-1-benzyl-4-\{S\}-2-[3-cyclopropyl-3-(2-isopropyl-thiazol-4-ylmethyl)-ureido]-3-methyl-butyrylamino]-3-hydroxy-5-phenyl-pentyl-carbamic acid thiazol-5-ylmethyl ester] in ethanol/water mixture (50:50, v/v). After a brief mixing, 850 μl of ethyl acetate and hexane (9:1, by volume) was added to the sample. After mixing and centrifugation, 500 μl of the organic extract was transferred to a clean 96-well plate and evaporated to dryness under a stream of dry nitrogen at room temperature. The samples were reconstituted with 300 μl of mobile phase for LC/MS analysis.

M2 and IS were separated on a 50 × 3 mm 2.7-μm Ascentis Express C18 (Supelco, Bellefonte, PA) column with an ACN 0.1% aqueous formic acid mobile phase containing 0.025% trifluoroacetic acid (~60-40, by volume) at a flow rate of 0.6 ml/min. Analysis was performed on a Sciex API 3000 Triple-Quadropole Mass Spectrometer with a turbo-ionspray interface (Sciex, Framingham, MA). Analyses were ionized in the positive ion mode with a source temperature of approximately 450°C. Detection was in the multiple reaction monitoring mode at m/z 782.3 → 587.2 for M2 and m/z 747.3 → 322 for IS. M2 and IS peak areas were determined using Sciex TurboQuan SOFTWARE. The plasma drug concentration of each sample was calculated by least-squares linear regression analysis of the peak area ratio (parent/IS) of the spiked plasma standards versus concentration.

Pharmacokinetic Calculations

Plasma concentration-time radioactivity data were analyzed with SAS software (version 9.2; SAS Institute Inc., Cary, NC). Maximum plasma concentration (C_{max}), the time at which C_{max} was achieved (T_{max}), concentration-time AUC from time 0 to the last measurable time point for total radioactivity, [14C]-paritaprevir, ritonavir and its metabolites in plasma were estimated. AUC from time 0 to infinity, t_{1/2}, for total radioactivity, and [14C]paritaprevir in plasma were also calculated.

In Vitro Studies

Human Fecal Incubations. Fresh fecal samples were collected from two healthy male subjects. Aliquots of the specimen (approximately 1 g) were placed into preweighed 15-ml centrifuge tubes with screw caps. The sample tubes were kept in an AnaeroPouch (Mitsubishi Gas Chemical Company, Tokyo, Japan) (Delaney and Onderdonk, 1997; Van Horn et al., 1997) with a PouchAnaero Anaerobic Gas Generating System and an RT AnaeroIndicator (Mitsubishi Gas Chemical Company) before transferring to a CO₂-filled Aldrich AtmosBag (glove bag; Sigma-Aldrich) that was connected to a CO₂ cylinder to create an anaerobic environment. An RT AnaeroIndicator was placed inside the bag to monitor the anaerobic conditions. Dulbecco’s phosphate-buffered saline was degassed by bubbling nitrogen gas through the solution overnight. The fecal samples were diluted with the Dulbecco’s phosphate-buffered saline to a concentration of ~100 mg/ml under a CO₂ atmosphere. The samples were mixed by vortexing to break up solid matter. An aliquot of fecal homogenate (1.5 ml) was added to the 2-ml Corning cryogenic vials containing [14C]paritaprevir and ritonavir at the final concentrations of 20 μM paritaprevir and 10 μM ritonavir. The vials were capped, vortexed, and placed in an AnaeroPouch with a PouchAnaero anaerobic gas-generating system and an RT AnaeroIndicator. The pouches were sealed before being removed from the glove bag. The pouches containing sample vials were placed in a 37°C water bath for 6–24, 48–hour. Anaerobic conditions were maintained throughout the study as monitored by the RT AnaeroIndicator. At the end of the incubation, the sample was mixed with 1.5 ml of ACN/methanol (3:1, v/v). After centrifugation, aliquots of supernatants were injected for HPLC-radiolow analysis or HPLC-MS-radiodetection analysis.

Incubations in Human Hepatocytes. The cryopreserved hepatocytes (10-donor pool) were obtained from Celsis In Vitro Inc. (lot #VR; Baltimore, MD). The hepatocyte suspension was diluted to 500,000 viable cells/ml. The incubations were conducted with or without chemical inhibitors. For the incubations with inhibitors, either ritonavir or 1-ABT was added to 0.5 ml of the diluted hepatocyte suspensions, at a final concentration of 1 μM or 1 mM for ritonavir or 1-ABT, respectively. The plates were incubated for 30 minutes at 37°C. [14C]Paritaprevir was then added to the pretreated hepatocyte suspension solution, at a final concentration of 1 μM, and incubated for 4 and 24 hours at 37°C. At the end of incubation, the 1-ml mixture of ACN/ethanol (50:50, v/v) was added to quench the reaction. Solutions were mixed thoroughly, spun down, and stored at −20°C until analysis.
Incubations in Simulated Intestinal Fluids. The simulated intestinal fluids (SIFs) were prepared by dissolving SIF powders (Biorelevant, Croydon, Surrey, UK) into a phosphate-buffered solution (pH 6.52) for fasted-state SIF (FaSSIF), or into a phosphate-buffered solution (pH 5.00) for fed-state SIF (FeSSIF). [14C] Paritaprevir was added to the FaSSIF or FeSSIF solutions to a final concentration of 10 μM. The solutions were incubated for 4, 8, and 24 hours at 37°C; aliquots of the solutions were subjected to HPLC-radioflow analysis.

Results
Excretion of Radioactivity
After a single oral dose of [14C]paritaprevir (200 mg and 100 μCi, with 100 mg of ritonavir) to four healthy volunteers, the excretion of radioactivity in urine and feces from all the subjects was measured up to 192 hours postdose. The mean cumulative percentage of the radioactive dose recovered in urine and feces was 96.5% over the 192-hour study, with recovery in individual subjects ranging from 96.0% to 96.9%. Most of the administered radioactivity was recovered in the first 120 hours postdose (90.5%). The radioactivity was excreted primarily through fecal elimination (mean, 87.8% of dose). Renal excretion was relatively minor (mean, 8.76% of dose).

Pharmacokinetic Data Analysis
The pharmacokinetic parameters for paritaprevir, ritonavir, and total radioactivity are summarized in Table 1. The concentration of total radioactivity was measured by LSC, expressed in nanogram-equivalents per gram. The concentrations of paritaprevir and ritonavir were determined using a validated LC-MS² bioanalytical method, expressed in nanograms per milliliter. Paritaprevir and total radioactivity T_max occurred approximately 3–4 hours after a single oral dose. The mean C_max for the paritaprevir and total radioactivity were 886 ng/ml and 1155 ng-eq/g, respectively. The concentrations of paritaprevir and total radioactivity apparently declined in parallel after reaching the peak concentration (Fig. 3). The AUC for concentration at time 0 to the last time point (AUC₀–last) values for the parent drug and total radioactivity were 4610 ± 3440 ng·h/ml and 4960 ± 3600 ng·eq·h/g, respectively. The AUC₀–last of paritaprevir measured by the LC-MS bioanalytical method represents the AUC for greater than 90% of total plasma radioactivity, suggesting that paritaprevir is the primary component in circulation.

Metabolite Profiles of [14C]Paritaprevir in Excreta and Circulation
Plasma. Representative HPLC radiochromatograms of [14C]-paritaprevir and its metabolites in pooled human plasma are shown in Fig. 4. [14C]Paritaprevir is the predominant component in human plasma, and metabolites M2 and M29 are present as minor components. Low levels of M13 were also detected, but only in the 2-hour pooled sample. In addition, trace levels of metabolites M3 and M6 are detected only by LC-MS analysis. The average percentage of plasma radioactivity for [14C]paritaprevir and its metabolites in the time point-weighted AUC₀–168 hours pooled plasma (Hamilton pooling) is tabulated in Table 2. Unchanged parent drug represented an average of 90.1% of radioactivity in the pooled plasma, and metabolite M2 accounted for ~6.5% of drug-related material in plasma. Other metabolites include M29 (2.1% of total radioactivity in plasma) and M3 (1.2% of total radioactivity in plasma).

Urine and Feces. Chromatographic evaluation of urine samples showed similar metabolites identified in human plasma, including paritaprevir, M2, M13, and M29. After 72 hours postdose to the last collection time point at 144–168 hours, M13 was the only radiochemical component detected in urine. The representative HPLC radiocchromatogram of pooled human urine is shown in Fig. 5A). M13 was the most significant component in urine throughout the entire sample collection period, accounting for 8.6% of the dose; unchanged levels of the parent drug and other metabolites were present at low to trace levels with respect to the administered dose. The mean quantification results for paritaprevir and metabolites in urine and feces, expressed as percentages of the administered radioactive dose, are tabulated in Table 3.

The representative HPLC radiocchromatogram of pooled human feces is shown in Fig. 5B. Paritaprevir was extensively degraded in feces. Unchanged parent drug levels accounted for only 1.1% of the total dose, whereas M29 was the most abundant radiochemical component in feces, accounting for 59.9% of total dose, followed by M2 (8.6%) and coeluting metabolites M3/M18 (7.5%). Other minor radiochemical components in feces included M24, M22, M23, M13, M14, M17, and M6 (each ≤3.3% of the dose) (Table 3). The proposed metabolic scheme for paritaprevir in humans is shown in Fig. 6.

LC-MS² Characterization of the Metabolites
As described in the Method for Metabolite Profiles and Identification section, metabolites of paritaprevir were characterized using a combination of positive ionization high-resolution full-scan MS and product ion scan (MS²) analyses. The structures of the metabolites M2 and M13 were confirmed against the reference materials, and the structures of other metabolites were proposed based on the high-resolution MS² fragmentation pattern analysis. The approximate retention time and key mass spectral fragmentation of paritaprevir and metabolites are listed in Table 4.

Paritaprevir. Parent drug yielded a protonated molecular ion ([M+H]⁺) at m/z 766.3003 (calculated mass m/z 766.3017, C₄₀H₄₄N₇O₈S⁺) in positive ion mode. The key fragments of M₁ (m/z 543.2383 (loss of phenanthridin-6-ol), m/z 559.2337 (loss of phenanthridin-6-ol and CO), m/z 571.2331 (base peak) and M₃ (m/z 422.2187 (predicted molecular formula as C₃₇H₃₇N₅O₃)), a loss of CO from product ion at m/z 450). The CID spectrum and fragmentation pathway of paritaprevir was provided in Supplemental Materials.

Metabolite M2. M2 produced a protonated molecular ion at m/z 782.2949, which is 16 atomic mass units higher than that of the parent drug and is consistent with the chemical formula C₄₀H₄₄N₇O₈S⁺ (calculated mass m/z 782.2967, parent + O). The CID of M₂ (m/z 782) produced major fragment ions, including the base peak at m/z 587.2291 (loss of phenanthridin-6-ol), m/z 559.2337 (loss of phenanthridin-6-ol and CO), m/z 502.1757 (loss of pyrrole and H₂O from
fragment ion m/z 587), m/z 474.1804 (loss of pyrrolidine-2-carbaldehyde from m/z 569). Therefore, M6 was assigned as a hydroxylated metabolite, and hydroxylation possibly occurred at the hydrocarbon region in the macrocyclic ring.

**Metabolite M13.** M13, a polar metabolite, was observed at solvent front in LC-MS, with a protonated ion of m/z 579.2900, indicating the addition of two oxygen atoms to the parent drug (predicted formula C₂₀H₂₈N₃O₅S⁺). The major fragment ions were m/z 603.2240 (loss of phenanthridin-6-ol), m/z 575.2292 (loss of phenanthridin-6-ol and CO), and m/z 422.1748 (predicted formula as C₁₀H₁₂N₂O₃S⁺). M13 is tentatively assigned as a hydroxylated M2, where the hydroxylation likely occurs at the methylpyrazine moiety.

**Metabolite M6.** M6, present at a trace level in plasma, gave a protonated molecular ion of m/z 782.2950, indicating addition of one oxygen atom to the parent drug (predicted formula C₃₇H₃₉N₆O₈S⁺). The major fragment ions of M6 included m/z 587.2281 (loss of phenanthridin-6-ol), m/z 569.2170 (loss of phenanthridin-6-ol and H₂O), and m/z 559.2327 (loss of phenanthridin-6-ol and CO). MS² of m/z 587 produced a major fragment ion at m/z 569.2170 (−H₂O) and 474.1804 (loss of pyrrolidine-2-carbaldehyde from m/z 569). Therefore, M6 was assigned as a hydroxylated metabolite, and hydroxylation possibly occurred at the hydrocarbon region in the macrocyclic ring.

**TABLE 1**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cₘₐₓ</th>
<th>Tₘₐₓ</th>
<th>AUC₀−₉₉₉</th>
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<td></td>
<td>ng-eq/g</td>
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<td>3.5 ± 1.9</td>
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<td>Paritaprevir</td>
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</tbody>
</table>

AUC₀−₉₉₉: AUC from time 0 to infinity.⁺Total radioactivity Cₘₐₓ unit, ng-eq/g; AUC unit, ng-eq/h/g.

**Fig. 3.** Mean (S.D.) plasma concentration-time curves for paritaprevir (ng/ml) and total radioactivity (ng-eq/g) in male subjects administered a single 200-mg oral dose of [¹⁴C]paritaprevir, coadministered with a single 100-mg oral dose of [¹⁴C]paritaprevir, coadministered with a single 100-mg oral dose of ritonavir (n = 4).

**Fig. 4.** Representative HPLC radiochromatograms of paritaprevir and its metabolites in human plasma at 2 hours (above) and 12 hours (below) after a single 200-mg oral dose of [¹⁴C]paritaprevir, coadministered with a single 100-mg oral dose of ritonavir to healthy male subjects.
Metabolites M14, M16, M18, M19, and M21. M14, M16, M18, M19, and M21 were detected only in feces at low levels; most of these were below radiochemical detection limit. These metabolites gave a protonated molecular ion at m/z 679.2863, which is consistent with the predicted formula of C_{37}H_{44}N_{7}O_{9}S^+ (calculated mass 679.2875). CID spectra of M14 and M19 showed the presence of fragment ion at m/z 468.2240 (loss of phenanthridine-diol), indicating that hydroxylation occurred at phenanthridine moiety. The hydroxylation position for M16, M18, and M21 was not further characterized due to the fact that the overall abundance of these metabolites is low in feces.

Metabolites M15, M17, and M20. M15, M17, and M20 were also exclusively detected in feces at trace to low levels. M15, M17, and M20 all gave protonated molecular ion at m/z 798.2894, which is consistent with the predicted formula of C_{40}H_{44}N_{7}O_{9}S^+ (calculated mass 798.2896). CID spectra of M15 and M19 showed the presence of fragment ion at m/z 603.2228 (loss of phenanthridin-6-ol) and 585.2125 (loss of H_2O). The presence of fragment ion at m/z 603.2240 (as a result of the loss of phenanthridin-6-ol and methylpyrazine-2-carboxylic acid) and m/z 438.1691 (as a result of the loss of phenanthridin-6-ol and methylpyrazine-2-carboxamide) and m/z 420.1585 (loss from ion m/z 438) suggests that M15 has the hydroxylation likely at macrocyclic region. M17 gave CID key fragment ions at m/z 603.2240, 575.2294 (loss of phenanthridin-6-ol and CO), and 422.1750. M17 is tentatively assigned as a dihydroxylate metabolite. M20 produced CID key fragment ions at 587.2277 (loss of phenanthridine-diol), 559.2326 (loss of phenanthridine-diol and H_2O), and 422.1741. M20 is tentatively the hydroxylate metabolite of M2 with hydroxylation at phenanthridine moiety.

**Metabolites M22 and M23.** Metabolite M22 and M23 were only detected in feces; both gave the protonated molecular ion at m/z 697.2965, which is consistent with the predicted formula of C_{32}H_{35}N_{4}O_{5}S^+ (calculated mass 697.2980), indicating the addition of one oxygen atom and one cysteine to the parent drug. CID of M15 produced key fragment ions at m/z 603.2228 (loss of phenanthridin-6-ol) and 585.2125 (loss of H_2O from ion m/z 603). The presence of fragment ion at m/z 697.2965 (loss of phenanthridine-diol and H_2O), and 690.2369 (loss of phenanthridine-6-ol and H_2O) and 692.2369 (loss of phenanthridine-6-ol and H_2O) and 764.2854 (loss of H_2O and cysteine), and 764.2854 (loss of H_2O and cysteine), and 764.2854 (loss of H_2O and cysteine), and 885.3042 (−H_2O), 782.2951 (−cysteine), 764.2854 (loss of H_2O and cysteine), and 690.2369 (loss of phenanthridine-6-ol and H_2O). The presence of fragment ion at m/z 690.2369 indicates that oxidation and cysteine conjugation occurs at the macrocyclic olefinic linker. Metabolite M24. Metabolite M24 was only observed in feces; it gave the protonated molecular ion at m/z 390.3138, which is consistent with the chemical formula C_{32}H_{35}N_{4}O_{5}S^+ (calculated mass 390.3164), indicating the possible addition of one oxygen atom and one cysteine to the parent drug. The major fragment ions were 885.3042 (−H_2O), 782.2951 (−cysteine), 764.2854 (loss of H_2O and cysteine), and 690.2369 (loss of phenanthridine-6-ol and H_2O). The presence of fragment ion at m/z 690.2369 indicates that oxidation and cysteine conjugation occurs at the macrocyclic olefinic linker.

**Metabolite M25.** Metabolite M25 was only observed in feces; it gave the protonated molecular ion at m/z 646.2683, which is consistent with the predicted formula of C_{31}H_{35}N_{4}O_{5}S^+ (calculated mass 646.2694). M25 is proposed to be an amide hydrolysis metabolite from the parent drug with a loss of methylpyrazine-2-carboxylic acid.

**Metabolite M26.** Metabolite M26 was observed only in feces; it gave the protonated molecular ion at m/z 543.2591, which is consistent with the predicted formula of C_{29}H_{35}N_{4}O_{5}S^+ (calculated mass 543.2602). M26 is proposed to be an amide hydrolysis metabolite from M29 with a loss of methylpyrazine-2-carboxylic acid.

**Metabolite M2 in Human Plasma at Steady State**

As discussed in the previous section, metabolite M2 is the main metabolite in circulation, representing approximately 6.5% after a single oral dose of [14C]paritaprevir/ritonavir. The plasma

---

**TABLE 2**

Average percentage of radioactivity in time point-weighted AUC_{0–12h} pooled human plasma (n = 4)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Radioactivity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paritaprevir</td>
<td>90.1</td>
<td>13.0</td>
</tr>
<tr>
<td>M2</td>
<td>6.5</td>
<td>9.4</td>
</tr>
<tr>
<td>M3</td>
<td>1.2</td>
<td>2.4</td>
</tr>
<tr>
<td>M29</td>
<td>2.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

---

**TABLE 3**

Percentages of excretory metabolites of in humans after administration of a single 200/100 mg oral dose of [14C]paritaprevir/ritonavir (n = 4)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Feces (0–192 h)</th>
<th>Urine (0–168 h)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Paritaprevir</td>
<td>87.8</td>
<td>8.76</td>
<td>1.15</td>
</tr>
<tr>
<td>M2</td>
<td>8.55</td>
<td>0.13</td>
<td>8.68</td>
</tr>
<tr>
<td>M6</td>
<td>0.78</td>
<td>ND</td>
<td>0.78</td>
</tr>
<tr>
<td>M3/M18*</td>
<td>7.47</td>
<td>ND</td>
<td>7.47</td>
</tr>
<tr>
<td>M13</td>
<td>1.67</td>
<td>8.57</td>
<td>10.2</td>
</tr>
<tr>
<td>M14</td>
<td>1.12</td>
<td>ND</td>
<td>1.12</td>
</tr>
<tr>
<td>M17</td>
<td>1.08</td>
<td>ND</td>
<td>1.08</td>
</tr>
<tr>
<td>M22/M23**</td>
<td>2.78</td>
<td>ND</td>
<td>2.78</td>
</tr>
<tr>
<td>M24</td>
<td>3.32</td>
<td>ND</td>
<td>3.32</td>
</tr>
<tr>
<td>M29</td>
<td>59.9</td>
<td>0.01</td>
<td>59.9</td>
</tr>
</tbody>
</table>

*ND, not detected.

**M3 and M18 were coeluting metabolites in fecal sample analysis.

**M22 and M23 were coeluting metabolites in fecal sample analysis.

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![Fig. 5](image-url)  
**Fig. 5.** Representative HPLC radiochromatograms of paritaprevir and its metabolites in human excreta, urine (A) and feces (B), after a single 200-mg oral dose of [14C]paritaprevir, coadministered with a single 100-mg oral dose of ritonavir to healthy male subjects.
concentration of metabolite M2 at the steady state was also determined after multiple oral dosing with paritaprevir/ritonavir in humans. Eight subjects received a 300-mg dose of paritaprevir, in combination with a 100-mg dose of ritonavir once daily for 14 consecutive days. Paritaprevir peak plasma concentrations averaged 6398 ng/ml (Table 5). The M2 peak plasma concentration was substantially lower, with a mean concentration of 21.7 ng/ml. Paritaprevir AUC values averaged 33595 ng/h/ml; M2 AUC values followed the trend noted in the Cmax values, with a mean value of 208.5 ng/h/ml. The M2 AUC values as a percentage of the A-1043422 AUC averaged 0.6%.

Metabolite Formation in In Vitro Fecal Incubation

Biotransformation of paritaprevir by human intestinal microflora was evaluated by incubations of [14C]paritaprevir (20 μM) with ritonavir (10 μM) in freshly prepared human fecal homogenate under anaerobic conditions. Figure 7 shows representative radiochromatograms of [14C]paritaprevir in human fecal homogenate incubations, indicating that paritaprevir rapidly undergoes extensive hydrolysis to form M29. M29 represented approximately 80% of the total radioactivity after a 6-hour incubation, and approximately 95% after a 24-hour incubation. In addition, the hydrolysis products M13 (representing 1–3% of the total radioactivity), and M26 (an amide hydrolysis product from M29 with a loss of M13) were also observed by LC-MS analysis. The results confirm that unchanged paritaprevir from biliary elimination and unabsorbed paritaprevir can readily be hydrolyzed to M29 and M13 in humans in vivo.

In Vitro Hydrolysis in SIFs

The stability of paritaprevir in SIFs was evaluated by incubating [14C]paritaprevir at 10 μM in either FaSSIF (pH 6.52) or FeSSIF (pH 5.0) solutions for 8 and 24 hours. HPLC radiochromatographic analysis indicated that paritaprevir was stable in both FaSSIF and FeSSIF solutions; no hydrolysis products were detected, indicating sulfonamide or amide hydrolysis is unlikely to occur chemically in human intestinal fluids either under fast or fed conditions.

Metabolite Formation in In Vitro Human Hepatocyte Incubation

The in vitro metabolite profile in human hepatocytes was evaluated by incubations of [14C]paritaprevir with or without P450 inhibitors (ritonavir or 1-ABT). As shown in Fig. 8, A and B, [14C]paritaprevir generates oxidative metabolites M2 and M3 (confirmed by LC-MS) in
human hepatocytes in the absence of chemical inhibitors. When either ritonavir or the pan-P450 inhibitor 1-ABT was coincubated with paritaprevir, [14C]paritaprevir is the only radioactive component detected, and the formation of oxidative metabolites (M2 and M3) was completely inhibited by these chemical inhibitors (Fig. 8C) by coincubation with ritonavir and (Fig. 8D) coincubation with 1-ABT. Note that hydrolysis metabolites (e.g., M29 and M13) were not detected in human hepatocytes with or without chemical inhibitors, suggesting that hydrolysis products M29 and M13 are not formed by hepatic enzymes.

**Discussion**

The mass balance, disposition, and metabolism of paritaprevir were evaluated in four healthy human subjects. After the administration of a single 200-mg oral dose of [14C]paritaprevir, coadministered with a single 100-mg oral dose of ritonavir, the mean total recovery of administered radioactive dose was 96.5%, with recovery in individual subjects ranging from 96.0 to 96.9%. The majority of the administered radioactive dose (87.8%) was excreted in feces. Renal elimination plays a minor role, since the radioactivity recovered in urine accounted for only 8.8% of the dose, indicating that paritaprevir and metabolites are primarily eliminated in humans through hepatobiliary and fecal elimination routes.

Metabolites of paritaprevir in plasma, urine, and feces were profiled using HPLC radioactivity detection, and the structures of metabolites were characterized using HPLC-high-resolution MS. Biotransformation of paritaprevir in humans involves the following: 1) P450-mediated oxidation on the olefinic linker, the phenanthridine group, the methylpyrazinyl group, or combinations thereof; and 2) amide hydrolysis at the acyl cyclopropane-sulfonamide moiety and the pyrazine-2-carboxyamide moiety. The P450-mediated oxidation of paritaprevir is greatly attenuated with ritonavir codosing via inhibition of the CYP3A-mediated first-pass metabolism.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. RT Min</th>
<th>Obs. [M+H]+</th>
<th>Calc. [M+H]+</th>
<th>Appm</th>
<th>Metabolite ID</th>
<th>Key Fragment Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paritaprevir</td>
<td>50.4</td>
<td>766.3003</td>
<td>766.3017</td>
<td>−1.8</td>
<td>Parent drug</td>
<td>571, 553, 543, 458, 450, 422, 406, 355</td>
</tr>
<tr>
<td>M29</td>
<td>39.1</td>
<td>663.2916</td>
<td>663.2926</td>
<td>−1.5</td>
<td>Sulfonamide hydrolysis</td>
<td>468, 450, 440, 422, 303</td>
</tr>
<tr>
<td>M6</td>
<td>37.9</td>
<td>782.2949</td>
<td>782.2967</td>
<td>−2.3</td>
<td>Parent drug + O</td>
<td>587, 559, 502, 474, 422, 327</td>
</tr>
<tr>
<td>M3</td>
<td>35.8</td>
<td>782.2951</td>
<td>782.2967</td>
<td>−2.1</td>
<td>Parent drug + O</td>
<td>764, 618, 587, 569, 559, 541, 474</td>
</tr>
<tr>
<td>M13</td>
<td>−3</td>
<td>139.0498</td>
<td>139.0502</td>
<td>−3.1</td>
<td>Parent drug + 2O</td>
<td>603, 575, 518, 490, 422</td>
</tr>
</tbody>
</table>

**Fecal-specific metabolites**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. RT Min</th>
<th>Obs. [M+H]+</th>
<th>Calc. [M+H]+</th>
<th>Appm</th>
<th>Metabolite ID</th>
<th>Key Fragment Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>M14</td>
<td>36.0</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.8</td>
<td>Parent drug + O</td>
<td>468, 440, 422, 373, 355, 303</td>
</tr>
<tr>
<td>M15</td>
<td>34.1</td>
<td>798.2894</td>
<td>798.2916</td>
<td>−2.8</td>
<td>Parent drug + 2O</td>
<td>780, 603, 585, 518, 438, 420</td>
</tr>
<tr>
<td>M16</td>
<td>33.1</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.8</td>
<td>Parent drug + O</td>
<td>484, 466, 456, 438, 371, 303</td>
</tr>
<tr>
<td>M17</td>
<td>32.8</td>
<td>782.2949</td>
<td>782.2967</td>
<td>−2.1</td>
<td>Parent drug + 2O</td>
<td>603, 575, 557, 518, 490, 422, 327</td>
</tr>
<tr>
<td>M18</td>
<td>32.5</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.8</td>
<td>Parent drug + O</td>
<td>484, 466, 456, 438, 371, 303</td>
</tr>
<tr>
<td>M19</td>
<td>31.9</td>
<td>798.2949</td>
<td>798.2967</td>
<td>−2.8</td>
<td>Parent drug + O</td>
<td>468, 450, 440, 422, 355, 303, 258</td>
</tr>
<tr>
<td>M20</td>
<td>30.1</td>
<td>798.2949</td>
<td>798.2967</td>
<td>−2.8</td>
<td>Parent drug + 2O</td>
<td>587, 559, 502, 474, 422</td>
</tr>
<tr>
<td>M21</td>
<td>28.9</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.8</td>
<td>Parent drug + O</td>
<td>661, 617, 484, 466</td>
</tr>
<tr>
<td>M22</td>
<td>22.1</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.4</td>
<td>Parent drug + O + H₂O</td>
<td>Accurate mass only</td>
</tr>
<tr>
<td>M23</td>
<td>21.7</td>
<td>679.2863</td>
<td>679.2875</td>
<td>−1.4</td>
<td>Parent drug + O + H₂O</td>
<td>679, 468, 450, 440, 303</td>
</tr>
<tr>
<td>M24</td>
<td>18.9</td>
<td>903.3148</td>
<td>903.3164</td>
<td>−1.8</td>
<td>Parent + O + cysteine</td>
<td>885, 867, 782, 764, 690, 569, 541, 457, 422</td>
</tr>
<tr>
<td>M25</td>
<td>36.1</td>
<td>646.2683</td>
<td>646.2694</td>
<td>−1.7</td>
<td>Amide hydrolysis</td>
<td>525, 497, 451, 423, 406, 345, 330, 302</td>
</tr>
<tr>
<td>M26</td>
<td>23.9</td>
<td>543.2591</td>
<td>543.2602</td>
<td>−2.0</td>
<td>Amide hydrolysis of M29</td>
<td>348, 320, 304, 302, 287</td>
</tr>
</tbody>
</table>

Approx., approximately; Calc., calculated; ID, identification; Obs., observed; RT, retention time.

#Observed only in fecal samples.

**TABLE 5**

Pharmacokinetics of paritaprevir and metabolite M2 after multiple oral dosing with paritaprevir/ritonavir in humans (day 14)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cmax</th>
<th>Tmax</th>
<th>AUC₀⁻₂₄</th>
<th>AUC₀⁻₂₄%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>h</td>
<td>ng/h/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paritaprevir</td>
<td>6398 (4036)</td>
<td>3</td>
<td>33595 (19380)</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>21.7 (17.3)</td>
<td>5</td>
<td>208.5 (138.5)</td>
<td>0.6 (0.3)</td>
</tr>
</tbody>
</table>

Values are given as mean (S.D.), unless otherwise indicated. AUC₀⁻₂₄, AUC from time 0 to 24 hours.

Fig. 7. Representative HPLC radiochromatograms of 20 μM [14C]paritaprevir with 10 μM ritonavir after incubations in fresh human fecal homogenate for 0 hours (A) and 6 hours (B).
Of the total radioactivity excreted in feces, the acyl sulfonamide hydrolysis product M29 was the primary radiochemical component (~60% of dose), followed by the oxidative metabolite M2 (8.6% of dose). Paritaprevir recovered in feces and urine represented 1.2% of a dose. The amide hydrolysis product M13 was the primary component found in human urine, accounting for 8.6% of a dose. In vitro incubations of [14C]paritaprevir in human fecal homogenate showed that paritaprevir is extensively hydrolyzed to form primarily M29 (>80% of radioactivity in a 6-hour incubation); M13 to much less of an extent (~1–3% of radioactivity); and M26, indicating the important role of human intestinal microflora in amide hydrolysis of paritaprevir. Fecal degradation of paritaprevir has also been observed in preclinical toxicology species (unpublished data). An in vitro stability assessment of [14C]paritaprevir in SIFs indicated that [14C]paritaprevir is chemically stable in human intestinal fluids under either fasted or fed conditions. In addition, the absence of hydrolysis products (M29 and M13) in in vitro human hepatocyte assays with or without chemical inhibitors (ritonavir or 1-ABT) suggests that the formation of hydrolysis products M29 and M13 is not mediated by hepatic enzymes.

After a single dose of [14C]paritaprevir with ritonavir, [14C]-paritaprevir was the major component in plasma, and five minor metabolites (M2, M3, M6, M13, and M29) were identified. Unchanged parent drug levels represented an average of 90.1% of radioactivity in plasma, and metabolite M2 accounted for ~6.5% of drug-related material in plasma. Other metabolites include M29 and M3 (2.1% and 1.2%, respectively, of total radioactivity in plasma). Steady-state concentrations of M2 in plasma samples obtained from subjects receiving multiple doses of 300/100 mg paritaprevir/ritonavir further confirm that there are no major circulating metabolites in human plasma. M2 is a nonreactive stable metabolite that, presumably, is likely formed through the epoxide intermediate. Since the overall metabolism contribution to the elimination of paritaprevir is minimal, the safety risk associated with the M2 metabolite and its formation pathway is remote. Clinically concomitant administration of strong CYP3A4 inhibitors or inducers may cause a modest increase or decrease in paritaprevir and ritonavir systemic exposures. Detailed discussion of potential drug-drug interaction mechanisms involving paritaprevir/ritonavir and other components in the DAA regimen is reported elsewhere (M. Shebley, D. Bow, J. Liu, O. Kavetskaia, J. Sydor, S. M. de Morais, V. Fischer, and M. Nijsen, manuscript in preparation).

In summary, the overall disposition and metabolism of 200 mg of [14C]paritaprevir codosed with 100 mg of ritonavir in healthy volunteers was investigated. The overall study objectives were met, with good recovery of the radioactivity dose from all subjects. The mass balance results confirm that orally administered paritaprevir is primarily eliminated by the biliary-fecal route. Paritaprevir is extensively degraded through microflora-mediated hydrolysis. The structures of metabolites in circulation and excreta were elucidated, with proposed metabolic pathways of CYP3A4-mediated oxidation, which is greatly inhibited by coadministered ritonavir and microflora-mediated hydrolysis.

Acknowledgments

The authors thank Rich Voorman for contributing to the study planning and valuable discussion, and Seble Wagaw for preparation of M2.

Authorship Contributions

Participated in research design: Shen, Menon, Kavetskaia, Fischer.
Conducted experiments: Serby, Zhang, Marsh.
Contributed new reagents or analytic tools: Serby, Reed.
Performed data analysis: Shen, Serby, Marsh, Khatri, Menon.
Wrote or contributed to the writing of the manuscript: Shen, Serby, Reed, Lee, Zhang, Marsh, Khatri, Menon, Kavetskaia, Fischer.
References


References


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