SPECIES DIFFERENCES IN THE FORMATION OF VABICASERIN CARBAMOYL GLUCURONIDE

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ABSTRACT

Vabicaserin is a potent 5-HT$_2C$ full agonist with therapeutic potential for a wide array of psychiatric disorders. Metabolite profiles indicated that vabicaserin was extensively metabolized via carbamoyl glucuronidation following oral administration in humans. In the present study, the differences in the extent of vabicaserin carbamoyl glucuronide (CG) formation in humans and in animals used for safety assessment were investigated. Following oral dosing, the systemic exposure ratios of CG to vabicaserin were about 12 and up to 29 in monkeys and humans, respectively, and the ratios of CG to vabicaserin were about 1.5 and 1.7 in mice and dogs, respectively. These differences in systemic levels of CG are likely related to species differences in the rate and extent of CG formation and elimination. While CG was the predominant circulating metabolite in humans, a major metabolite in mice, dogs, and monkeys; it was a relatively minor metabolite in rats, where oxidative metabolism was the major metabolic pathway. Although the CG was not detected in plasma or urine of rats, about 5% of the dose was excreted in bile as CG in the 24 hr collection post-dose, indicating the rat had the metabolic capability of producing the CG.

In vitro, in a CO$_2$-enriched environment, the CG was the predominant metabolite in dog and human liver microsomes, a major metabolite in monkey and mice and only a very minor metabolite in rats. Carbamoyl glucuronidation and hydroxylation had similar contributions to vabicaserin metabolism in mouse and monkey liver microsomes. However, only trace amounts of CG were formed in rat liver microsomes and other metabolites were more prominent than the CG. In conclusion, significant differences in the extent of formation of the CG were observed among the various species examined. The exposure ratios of CG to vabicaserin were highest in humans followed by monkeys then mice and dogs and lowest in rats, and the in vitro metabolite profiles generally correlated well with the in vivo metabolites.
**Introduction**

Vabicaserin is a potent 5-HT$_{2C}$ full agonist and demonstrates in vitro functional selectivity for 5-HT$_{2C}$ over 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Dunlop et al., 2006). Vabicaserin is effective in several animal models that are predictive of antipsychotic activity, with an atypical antipsychotic profile (Marquis et al., 2006). Administration of vabicaserin decreases nucleus accumbens dopamine without affecting striatal dopamine, which is indicative of mesolimbic selectivity (Marquis et al., 2006, Wacker and Miller 2008). This profile is consistent with potential efficacy in the treatment of the psychotic symptoms of schizophrenia with decreased liability for extrapyramidal side effects. In addition, chronic administration of vabicaserin significantly decreases the number of spontaneously active mesocorticolimbic dopamine neurons, without affecting nigrostriatal dopamine neurons (Marquis et al., 2006), consistent with the effects of atypical antipsychotics.

The safety, tolerability, pharmacokinetics and pharmacodynamics were assessed in healthy subjects following single ascending oral doses of 2 to 500 mg (Posener JA et al., 2007) and in subjects with schizophrenia following dose of 100 to 250 mg every 12 hours (BID)) for 10 days (Mako B et al., 2008). In both studies, vabicaserin was safe and well-tolerated following single and multiple dose administration. The vabicaserin peak concentrations (C$_{max}$) and area under the concentration-time curve (AUC) values generally increased linearly over the dose ranges. Less than 1 % of the administered dose was excreted in the urine as unchanged drug in these studies. A carbamoyl glucuronide (CG) was identified as the predominant human metabolite, exceeding systemic concentrations of vabicaserin by approximately 60 times. Carbamoyl glucuronides are reported to be formed by the reaction of an amine with carbon dioxide, forming a carbamic acid and subsequent conjugation with glucuronic acid. Amino acids (Morrow et al., 1974) and other
amines (Greenaway and Whatley, 1987; Delbressine et al., 1990) may spontaneously react with CO₂ to form carbamic acids, which is reversible and does not require enzymatic catalysis. The carbamic acids may react with uridine 5'-diphosphoglucuronic acid (UDPGA) under the catalysis of UDP-glucuronosyltransferases (UGTs) to yield glucuronides, which block the dissociation of CO₂ from parent drugs. Formation of carbamoyl glucuronides has been reported for primary amines such as mexiletine, mofegiline, rimantadine and tocainide, and secondary amines such as sertraline, carvedilol and N-dealkylation metabolites of benzazepine and ropinirole (Schaefer, 2006). Carbamoyl glucuronidation was the major metabolic pathway for tocainide and mexiletine. In healthy human subjects, about 25-40% of administered tocainide was excreted as the carbamoyl glucuronide in urine (Gipple et al., 1982). Approximately 30% of administered mexiletine was excreted as a carbamoyl glucuronide in human urine (Senda et al., 2003).

Species differences in the formation of various carbamoyl glucuronides have been reported (Elvin et al., 1980; Gipple et al., 1982; Beconi et al., 2003). Beconi et al. reported that circulating levels of the carbamoyl glucuronide of a dipeptidyl peptidase IV inhibitor were significant in dogs, but present only in trace amounts in rats and monkeys (Beconi et al., 2003). While formation of tocainide carbamoyl glucuronide was a major metabolic pathway in humans (Elvin et al., 1980), the pathway was insignificant in non-clinical models in vivo (Gipple et al., 1982). The carbamoyl glucuronide of SK&F-104557, an N-despropyl metabolite of ropinirole, was observed in monkey plasma and urine, and in human urine, but was not observed in plasma or urine from mice and rats following an oral or intravenous administration (Ramji et al., 1999). The present study examined the in vivo and in vitro species differences in vabicaserin carbamoyl glucuronidation in healthy male human subjects and in animal models (mice, rats, dogs and monkeys) used in safety testing.
Materials and Methods

Materials. [14C]Vabicaserin hydrochloride was synthesized by the Radiosynthesis Group, Chemical Development, Wyeth Research at Pearl River, NY. The radiochemical purity of [14C]vabicaserin was 98.9% and the chemical purity was 99.9% by UV detection. The specific activity of the [14C]vabicaserin was 222.9 μCi/mg as a hydrochloride salt. Non-labeled vabicaserin hydrochloride, with a chemical purity of 98.6%, was synthesized by Wyeth Research at Pearl River, NY. Vabicaserin carbamoyl glucuronide (CG) was synthesized by Chemical Development at Wyeth Research at Montreal, Canada, and had a purity of 95.5%. The chemical structures of [14C]vabicaserin and CG are shown in Figure 1. Liver microsomes listed in Table 1 from CD-1 mice, Sprague-Dawley rats, beagle dogs and cynomolgus monkeys were obtained from In Vitro Technologies (Baltimore, MD). Pooled human liver microsomes were prepared from livers of 2 male and 4 female subjects purchased from IIAM (Exton, PA). Ultima Gold and Ultima Flo M scintillation cocktails were purchased from Perkin Elmer Life and Analytical Sciences (Waltham, MA). Other chemicals of analytical grade or better, and solvents of high performance liquid chromatography (HPLC) grade were obtained from EMD Chemicals (Gibbstown, NJ) or Mallinckrodt Baker, Inc. (Phillipsburg, NJ).

Human Study. This study was a randomized, double-blind, placebo-controlled, inpatient, sequential-group trial of ascending single doses of vabicaserin administered to healthy male subjects after an overnight fast of at least 10 h. The study was conducted at a single investigational site (Methodist Hospital, Philadelphia, PA). Oral doses of vabicaserin capsules ranging from 2 to 500 mg were administered to six healthy male subjects under fasting conditions. For metabolite analysis, plasma samples were collected from subjects receiving doses of 50, 200, 300 and 500 mg at approximately 2 h pre-dose and at 6, 12 and 24 h post-dose.
Urine specimens were collected at intervals of 0-4, 4-12, 12-24, and 24-48 h. Samples were stored at approximately -70°C until analysis.

The protocol, the investigator’s brochure, and the informed consent forms (ICFs) were submitted to the study site institutional review board (IRB) for review and written approval. Subsequent amendments to the protocols and/or any revisions to the ICFs were submitted for IRB review and written approval. This study was conducted in accordance with ethical principles that have origins in the Declaration of Helsinki and in any amendments that were in place when the study was conducted. This study were also designed and performed in compliance with Good Clinical Practice (GCP). Written informed consent was obtained from all subjects before their enrollment.

**Animal Studies.** For metabolism studies in mice, rats and dogs, radio-labeled doses were used. Male and female CD-1 mice and Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). The dose vehicle for mice and rats contained 2% (w/w) Tween 80 and 0.5% methylcellulose in water. Non-fasted male and female mice weighing from 27.8 to 33.8 g at the time of dosing were given a single 50 mg/kg (~300 μCi/kg) dose of vabicaserin at a volume of 20 mL/kg via intragastric gavage. Mice were kept in metabolic cages in groups of five. Non-fasted male rats weighing from 318 to 345 grams and female rats weighing from 227 to 255 g at the time of dosing were given a single 5 mg/kg (~300 μCi/kg) dose of vabicaserin at a volume of 2.5 mL/kg via intragastric gavage. Four bile-duct cannulated male rats weighing from 387 to 411 g and four bile-duct cannulated female rats weighing from 291 to 325 g at the time of dosing were non-fasted and were given a single 5 mg/kg (323 μCi/kg) dose of vabicaserin at a volume of 5.0 mL/kg via intragastric gavage. Rats were kept individually in metabolism cages.
Four male beagle dogs, weighing from 7.6 to 9.8 kg at the time of dosing, were from an in-house colony. About 11 mg of [14C]vabicaserin hydrochloride and 940 mg of non-labeled vabicaserin hydrochloride were dissolved in methanol and then evaporated under a nitrogen stream to dryness. Capsules (#2) were filled with accurate amounts (126.7-138.1 mg) of the mixed drug substance according to animal weights to give a dosage of 15 mg/kg (39 μCi/kg). The filled gelatin capsules were then enteric-coated manually. Each dog was given one enteric-coated capsule containing [14C]vabicaserin as the hydrochloride salt. Animals were fed two hours prior to dosing and were housed individually in metabolic cages.

Four male cynomolgus monkeys, weighing from 5.4 to 9.6 kg at the time of dosing, were from an in-house colony. Non-fasted monkeys were given a single 25 mg/kg dose of non-radiolabeled vabicaserin at a volume of 2 mL/kg via intragastric gavage. The vehicle was the same as used in mice and rats. Animals were housed individually in metabolic cages.

All animal housing and care was conducted in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facilities. Animal care and use for this investigation was approved by the Wyeth Institutional Animal Care and Use Committee. Animal rooms were maintained on a 12-hour light and dark cycle. Animals were provided food and water ad libitum. Blood samples were collected from mice (5/time point) and rats (3/time point) at sacrifice by cardiac puncture at 2, 4, 8 and 24 hr post-dosing from males, and at 2 and 8 hr from females. Blood samples of about 3 mL from the jugular vein of dogs and from the femoral trigon of monkeys were collected at 2, 4, 8 and 24 hr post-dosing from males, and at 2 and 8 hr from females. Potassium EDTA was used as the anticoagulant and plasma was immediately harvested from the blood by centrifugation at 4°C. Urine samples were collected from animals at 0-8 and 8-24 hr intervals. Only bile samples were collected from bile duct cannulated rats into
tubes on dry ice at 0-4, 4-8 and 8-24 hr intervals. All biological specimens were stored at approximately -70°C until analysis.

**Incubations with Liver Microsomes.** [14C]Vabicaserin (10 and 50 μM) was incubated for 20 minutes in a CO2-enriched environment with liver microsomes (0.5 mg/ml) in 0.5 mL of 50 mM bicarbonate buffer, pH 7.4, containing alamethicin (50 μg/mg protein) and magnesium chloride (10 mM), in the presence of an NADPH regenerating system and UDPGA (2 mM). The NADPH regenerating system consisted of glucose-6-phosphate (2 mg/mL), glucose-6-phosphate dehydrogenase (0.8 units/mL) and NADP+ (2 mg/mL). A mixture of [14C]vabicaserin and non-radiolabeled vabicaserin (1:3 or 1:5) in 20 μL water was added to the incubations. After mixing and pre-incubating for 3 minutes in a shaking water bath at 37°C, reactions were started by the addition of the cofactors. All incubations were performed in duplicate. Under conditions optimized for carbamoyl glucuronide formation and an incubation period of 20 minutes, metabolite formation was generally linear. Control assays were conducted under the same incubations conditions, but without cofactors. Incubations were stopped by the addition of 0.5 mL ice-cold methanol. Samples were vortex-mixed and denatured proteins were separated by centrifugation at 4300 rpm and 4°C for 10 minutes (Model T21 super centrifuge, Sorvall). The protein pellets were extracted with 0.5 mL of methanol. The supernatants were combined for each sample, mixed and evaporated to a volume of about 0.3 mL under a nitrogen stream in a TurboVap LV evaporator (Caliper Life Sciences, Hopkinton, MA). The concentrated sample was centrifuged and aliquots were radioassayed and analyzed by HPLC. With this method, greater than 90% of the radioactivity was recovered from the reaction mixture.

**Radioactivity Determination.** Aliquots of plasma (50 μL) and urine (100-200 μL) samples were analyzed for radioactivity with a Tri-Carb Model 3100 TR/LL LSC (Perkin Elmer) using
10 mL of Ultima Gold as the scintillation fluid. In-line radioactivity detection for HPLC was accomplished by a TopCount microplate reader or a Flo-One radioactivity detector (Perkin Elmer). For mouse and dog plasma samples, the effluent was collected at 20 second intervals into 96-well Lumaplates (Perkin Elmer). The plates were dried overnight in an oven at 40°C and analyzed by the TopCount NXT radiometric microplate reader. For other animal samples and in vitro incubations, a Flo-One β Model A525 radioactivity detector with a 250 μL flow cell was used for data acquisition.

**Preparation and Analysis of Mouse, Rat and Dog Samples.** Plasma and urine samples from mice, rats and dogs were analyzed by HPLC with radioactivity detection for metabolite profiles. Pooled plasma and urine samples were analyzed by LC/MS for metabolite characterization. Plasma concentrations of vabicaserin and CG were calculated by multiplying the total plasma radioactivity concentrations with the percentages of radioactivity associated with vabicaserin and CG peaks. Aliquots of plasma were mixed with two volumes of cold methanol, placed on ice for about 5 minutes and then centrifuged. The supernatant fluid was transferred to a clean tube and evaporated at 22°C under nitrogen in a TurboVap LV (Caliper Life Sciences) to a volume of about 0.3 mL. The concentrated extract was centrifuged, the supernatant volume measured and extraction efficiency was determined by analyzing duplicate 10 μL aliquots for radioactivity. An average of greater than 80% of plasma radioactivity was extracted by this method. An aliquot (50-200 μL) of the plasma supernatant was analyzed by HPLC with detection of radioactivity by either flow scintillation analysis or microplate reading as described above. Bile and urine samples were analyzed by HPLC with radiometric detection for metabolite profiles by direct injection.
A Waters model 2690 HPLC system (Waters Corporation, Milford, MA) with a built-in autosampler was used for analysis of animal samples. Separations were accomplished on a Phenomenex Luna C18(2) column (150 × 2.0 mm, 5 μm) (Phenomenex, Torrance, CA). The sample chamber of the autosampler was maintained at 4°C, while the column was at ambient temperature of about 20°C. For the analysis of in vivo samples, the HPLC mobile phase consisted of 10 mM ammonium acetate, pH 4.5 and acetonitrile, and was delivered at 0.2 mL/min. When a Flo-One radioactivity detector was used for data acquisition, the flow rate of Ultima Flow M scintillation fluid was 1 mL/min, providing a mixing ratio of scintillation cocktail to mobile phase of 5:1. Acetonitrile was maintained at 10% for 6 minutes, increased to 40% over 29 minutes, to 85% over 30 minutes and then maintained at 85% for another 5 minutes. For the analysis of in vitro incubations and mouse samples, methanol was used in place of acetonitrile. For the in vitro samples, methanol was maintained at 10% for 3 minutes, increased to 40% over 22 minutes, to 85% over 20 minutes and maintained at 85% for another 5 minutes.

Preparation and Analysis of Monkey and Human Samples. Human and monkey samples were analyzed by LC/MS for metabolite profiles and metabolite characterization, and by a non-validated LC/MS/MS method for concentrations of vabicaserin and CG as described below. The internal standard $d_8$-vabicaserin (25 μL of 400 ng/mL methanol solution) was added to 100 μL of the plasma samples, followed by the addition of 300 μL of acetonitrile. The samples were mixed and centrifuged at 16,000 rcf in an Eppendorf 5415C centrifuge (Brinkman Instruments Inc., Westbury, NY) for 10 min. The supernatant from each sample was transferred to a clean tube and evaporated to dryness under a stream of nitrogen in a TurboVap LV evaporator (Caliper Life Sciences). The residue was reconstituted with 50 μL of methanol followed by the addition of 150 μL of water. The sample was mixed and centrifuged, and the supernatant was analyzed by
LC/MS/MS analysis as described below. Standard curves were prepared with control plasma (100 μL) spiked with vabicaserin (10 μL) or synthetic CG (10 μL). The concentrations used for the standard curve ranged from 0.01 to 1,000 ng/mL of plasma for vabicaserin and 0.01 to 5,000 ng/mL of plasma for CG.

Urine samples were analyzed for vabicaserin and CG concentrations by direct injection after the addition of the internal standard d8-vabicaserin (25 μL of 400 ng/mL methanol solution) to 100 μL of the urine. Standard curves were prepared with control urine (100 μL) spiked with vabicaserin (10 μL) or synthetic CG (10 μL). The concentrations used for the standard curve ranged from 0.01 to 2,500 ng/mL of urine for vabicaserin and 0.01 to 10,000 ng/mL of urine for CG. A ten-fold dilution with control urine was made for some of the urine samples before they were prepared for analysis.
The LC/MS System consisted of a Thermo Surveyor HPLC (Thermo Scientific, San Jose, CA), including a Surveyor MS pump and autosampler. The LC conditions were the same as described above for radiolabeled in vivo samples. During LC/MS sample analysis, up to 3 min of the initial flow was diverted away from the mass spectrometer prior to evaluation of metabolites. The mass spectrometer used was a Finnigan TSQ Quantum triple quadrupole mass spectrometer (Thermo Scientific) equipped with an electrospray ionization interface and operated in the positive ionization mode. The spray voltage was 4.5 kV and the capillary temperature was 250°C. Q1 and Q3 mass resolutions were 0.8 and 0.6 Da width at half height, respectively. The collision gas pressure was 1.5 mtorr. The collision offset was 22 eV for vabicaserin and d8-vabicaserin and 25 eV for CG. For semi-quantitative analysis, transitions of m/z 229→186, 237→194 and 449→273 were used for selected reaction monitoring (SRM) of vabicaserin, d8-vabicaserin and CG, respectively.

**Liquid Chromatography/Mass Spectrometry (LC/MS) for Metabolite Identification.**

Waters model 2695 (Waters Corp., Milford, MA) and Agilent 1100 (Agilent Technologies, Palo Alto, CA) HPLC systems equipped with a diode array UV detectors were interfaced to the mass spectrometers described below for metabolite identification. UV spectral data were simultaneously recorded with mass spectral data. Separations were accomplished on a Luna C18(2) column (150 x 2.0 mm, 5 μm) (Phenomenex, Torrance, CA) coupled with a guard (4 x 2 mm) cartridge. The sample chamber of the autosampler was maintained at 4°C, while the column was maintained at 25°C. The mobile phase consisted of 10 mM ammonium acetate, pH 4.5 (A) and methanol (B) and was delivered at 0.2 mL/min. The linear gradient started at 5% B for the first 3 min, was held at 10% for the next 10 min, and increased to 20% by 25 min, 30% by 50 min, and 95% by 50 min where it was maintained for another 10 min.
Mass spectral data for vabicaserin metabolite identification were obtained with either a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters) or a Finnigan LCQ ion trap mass spectrometer (Thermo Scientific). Both mass spectrometers were equipped with an electrospray ionization source and operated in the positive ionization mode. Settings for each mass spectrometer were optimized to provide a structurally relevant range of product ions from MS/MS and MS² experiments. MassLynx (version 4.0, Waters) and Xcalibur (version 1.3, Thermo Scientific) software were used for collection and analysis of LC/MS data.
Results

In Vivo Formation of Vabicaserin Carbamoyl Glucuronide.

Representative plasma and urinary metabolite profiles in healthy male human subjects administered a single oral oral 500 mg dose of vabicaserin are shown in Figures 2 and 3 as summed mass chromatograms. Concentrations of CG in plasma were consistently higher than those for vabicaserin at 6, 12 and 24 h post-dose following single oral capsule doses ranging from 50 to 500 mg to healthy male subjects (Table 2). The estimated plasma AUC_{0-24} ratios for CG to vabicaserin ranged from 20 to 29. In general, the plasma concentrations of CG increased in a dose-related manner. As observed in plasma, the urinary concentrations of CG were greater than those for vabicaserin, with the concentration ratios ranging from 96 to 537 across the various doses and individual time intervals (Table 3). Less than 1% of the administered dose was estimated to be excreted in urine as unchanged drug, while the CG may account for as much as 50% or more of the dose in urine.

Following a single oral dose of [^{14}C]vabicaserin at 50 mg/kg, 5 mg/kg and 15 mg/kg, unchanged drug represented less than 19%, 20% and 35% of total plasma radioactivity at all time points examined in mice, rats and dogs, respectively (Table 4). The CG represented about 7-36% of plasma radioactivity in mice and 2-28% of plasma radioactivity in dogs, but was not detected in rat plasma after the single [^{14}C]vabicaserin dose (Table 4). However, the CG was observed in rat plasma following multiple-dose administration of vabicaserin at higher doses, and the CG was approximately 20 times less than vabicaserin based on steady-state AUC_{0-24} values (data not shown). The estimated plasma AUC_{0-24} ratios of CG to the parent drug was 1.5 and 1.7 in mice and dogs after the single [^{14}C]vabicaserin dose, respectively (Table 4). The plasma AUC_{0-24} ratios for the CG to vabicaserin at steady-state with doses used for safety assessment were less
for mice (0.2–0.6) and slightly higher for dogs (1.8–4.0) when compared with the single dose values (data not shown). The CG was detected in dog urine in similar amounts to the parent drug, although was not detected in mouse or rat urine after the single [14C]vabicaserin dose. Radioactivity in a 0-24 hr bile collection from rats receiving a 5 mg/kg [14C]vabicaserin dose accounted for 19 and 24% of the administered dose in males and females, respectively. While the CG was not detected in urine or feces of rats following a single oral administration, it represented an average of up to 30% of biliary radioactivity in male rats and 15% in female rats (Table 5), indicating that biliary elimination was a major excretion pathway for the CG in the rat. The absence of the CG in the feces may indicate that hydrolysis of the CG by intestinal flora occurred.

The in vivo animal metabolism data demonstrated that vabicaserin was extensively metabolized and that the amount of CG observed differed between species. Using AUC0-24 values, the CG represented less than 20% of the circulating radioactivity in mice, less than 10% in dogs and was not detectable in rats following a single oral administration of [14C]vabicaserin, indicating that in dogs and mice, other metabolites were as or more prominent than the CG. In rats, vabicaserin represented 20% or less of the plasma radioactivity, indicating that metabolites other than the CG were present.

In monkeys after a single oral 25 mg/kg dose of vabicaserin, the plasma concentrations of the CG exceeded those of vabicaserin at all time points (2 -24 hr) post-dose, although the amount of CG relative to vabicaserin decreased by 24 hours post-dose, with ratios of 17.5 at 2 hr and 1.7 at 24 hr (Table 6). The CG to vabicaserin AUC0-24 ratio of 12.1 indicated that the CG was a major metabolite in monkeys. A representative plasma metabolite profile based on the summed mass chromatogram showed the CG as a prominent metabolite relative to vabicaserin and the
presence of other metabolites (Figure 4). In urine, concentration ratios of the CG to the parent drug were 117, 262 and 38 for samples collected at 0-8, 8-24 and 24-48 hr, respectively (data not shown). Approximately 3% of the administered dose was excreted as the parent drug and CG in monkey urine in 0-48 hr post-dose.

**Formation of Vabicaserin Carbamoyl Glucuronide in Liver Microsomes.** When $[^{14}C]$vabicaserin was incubated with liver microsomes in bicarbonate buffer and a CO$_2$-enriched environment in the presence of both NADPH and UDPGA, under the initial rate conditions the turnover of vabicaserin was less than 10% in all incubations. Carbamoyl glucuronidation was the major metabolic pathway in dogs and humans, and a prominent pathway in monkeys and mice (Figure 5). Carbamoyl glucuronidation was only a very minor metabolic pathway in rats, with other oxidative pathways providing a greater contribution to the metabolite profile. Monkeys and mice exhibited similar in vitro metabolite profiles, with carbamoyl glucuronidation and other oxidative pathways both contributing to the overall metabolite profiles. However, the turnover of vabicaserin appeared to somewhat higher in monkeys compared to mice under the incubation conditions. The in vitro metabolite profiles were generally consistent with the in vivo pattern of CG formation across species.

**Metabolite Characterization.** Vabicaserin was characterized by LC/MS, which exhibited a $[M+H]^+$ at $m/z$ 229 and an HPLC retention time of about 69 min. The product ions of $m/z$ 229 mass spectrum for vabicaserin and the proposed fragmentation scheme are shown in Figure 6. Loss of NH$_3$ from $[M+H]^+$ yielded the product ion at $m/z$ 212. Loss of methyleneamine and ethylideneamine from $[M+H]^+$ generated product ions at $m/z$ 200 and 186, respectively. Subsequent loss of propene group from the cyclopentane ring generated product ions at $m/z$ 158
and 144, respectively. Loss of the cyclopentyl-methyleneamine group from [M+H]$^+$ generated the ion at $m/z$ 132.

**CG.** The CG metabolite had an HPLC retention time of about 75 min and formed a [M+H]$^+$ at $m/z$ 449. Figure 7 shows the product ions of $m/z$ 449 mass spectrum for the CG and the proposed fragmentation scheme. Loss of 176 Da from [M+H]$^+$ to generate the product ion at $m/z$ 273 and the presence of the ion at $m/z$ 113 indicated a glucuronide. Further loss of 44 Da (CO$_2$) from $m/z$ 273 generated $m/z$ 229, which was also the [M+H]$^+$ for vabicaserin. Product ions at $m/z$ 212 and 186 were also observed for vabicaserin. Confirmation of the identification was obtained from HPLC retention time and mass spectral data for the CG matching those of the synthetic carbamoyl glucuronide of vabicaserin.
Discussion

The extent of CG formation in humans differed from that in animals where circulating concentrations of the CG relative to vabicaserin in humans were up to 74 times higher and the AUC_{0-24} values were up to 29 times higher. Urinary concentrations of the CG also far exceeded those of vabicaserin based on metabolite profiles obtained by LC/MS using reference standards, indicating that urinary excretion was likely a major elimination route for the CG. Clearance of vabicaserin in healthy human male subjects was predominately by metabolism via carbamoyl glucuronidation, based on the plasma and urinary metabolite profiles. The extent of CG formation for vabicaserin in humans appears to be uncommon. While some primary and secondary amine containing drugs have been reported to have high turnover to their respective carbamoyl glucuronides, none produced systemic carbamoyl glucuronide exposures relative to parent as high as those observed with vabicaserin. A human metabolism and excretion study with radiolabeled vabicaserin is expected to provide data that will lead to further understanding of the carbamoyl glucuronide formation and overall metabolic disposition of this potential therapeutic agent, including excretion patterns.

In the non-clinical species, which included mice, rats, dogs and monkeys, vabicaserin was extensively metabolized. Unlike humans, the circulating levels of CG were only slightly higher than those of vabicaserin in mice and dogs, and much less in rats. In monkeys, the formation of the CG was higher than the other animal species, although still less than those in humans. It is noteworthy that in the species which exhibited lower levels of CG formation also exhibited oxidative pathways which contributed more to the overall metabolite profiles. These other pathways were most apparent in rats. In monkeys, metabolite profiles showed that vabicaserin
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was metabolized by carbamoyl glucuronidation and oxidative metabolism to an approximately similar extent. The circulating levels of the carbamoyl glucuronide of vabicaserin expressed in terms of CG/vabicaserin ratios were essentially absent in rats, highest in monkeys followed by dogs and mice. These differences in systemic levels of CG are likely related to the rate and extent of CG formation and elimination. The CG was not detected in rat plasma, however represented a significant portion of the biliary radioactivity accounting for 5% of the administered dose in the 24 hr bile collection, which could indicate that rats are able to produce the CG and apparently can effectively eliminate the metabolite, preventing measureable levels of the CG in plasma. In fact, the carbamoyl glucuronide of a GABA<sub>A</sub> receptor agonist, which was observed in rat bile, was not detected in rat plasma or urine (Shaffer et al., 2005). Recently, Shaffer et al reported that 68% of the oral dose of an α<sub>4</sub>β<sub>2</sub> nicotinic acetylcholine receptor partial agonist was detected as its carbamoyl glucuronide in rat bile, while the carbamoyl glucuronide was not observed in serum or urine (Shaffer 2009). They further demonstrated the indirect enterohepatic cycling of the parent drug via the carbamoyl glucuronide. The carbamoyl glucuronide of sitagliptin was reported to be a major metabolite in dog bile, even though it was not observed in urine (Beconi et al., 2007). Species differences in the formation of carbamoyl glucuronides have also been reported for other compounds as described in the Introduction and are not unusual.

In liver microsomes, under experimental conditions using a CO<sub>2</sub>-enriched environment optimized for carbamoyl glucuronide formation, species differences in carbamoyl glucuronidation were readily apparent. The CG was the predominant metabolite in dogs and humans, a major metabolite in monkeys and mice and only a very minor metabolite in rats.
From incubations using initial rate conditions for metabolite formation, the overall extent of CG formation appeared greater in dogs, monkeys and humans, less extensive in mice and lowest in rats. The distribution of metabolites in the chromatographic profiles appeared to indicate that both monkeys and mice produced similar metabolites including the CG. Rat, on the other hand, clearly exhibited metabolites other than the CG as more prominent. Although humans also formed metabolites in addition to the CG, these other metabolites were less prominent than the CG. Overall, these in vitro metabolite patterns were supportive of those observed in from the in vivo evaluations, and were generally predictive of the observed in vivo metabolites.

Carbamoyl glucuronides, in general, are known to be relatively stable metabolites. Unlike acyl glucuronides, which can undergo a number of reactions including non-enzymatic hydrolysis, rearrangement, and covalent binding to proteins (Spahn-Langguth and Benet, 1992; Fenselau, 1994), carbamoyl glucuronides are considered to be stable (Tremaine et al., 1989). Indeed, stability data from the various animal and human studies and the synthetic standard showed that the carbamoyl glucuronide of vabicaserin also was stable during the work up procedure and can be hydrolyzed only by incubation with β-glucuronidase (data not shown). Toxicity or covalent binding of carbamoyl glucuronides have not been reported. Therefore, formation of vabicaserin carbamoyl glucuronide in humans, even at concentrations far exceeding that of the parent drug, may not represent a potential safety concern. In addition, UGT enzymes are often considered high capacity enzyme systems compared to the cytochrome P450 enzymes, therefore, saturation leading to non-linear kinetics and/or drug drug interactions are not expected for vabicaserin.
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In conclusion, significant differences in the extent of CG formation were observed among the various species examined. While carbamoyl glucuronide was the predominant circulating metabolite in humans and a major metabolite in the other species except rat, oxidative metabolism also contributed to the metabolism of vabicaserin in all species to a varied extent, with rat exhibiting the highest level of oxidative metabolism. The exposure ratios of CG to vabicaserin were highest in humans followed by monkeys then mice and dogs and lowest in rats. The in vitro metabolite profiles were generally consistent with the in vivo metabolites observed in mice, rats, dogs, monkeys and in healthy human male subjects, this was particularly apparent for the CG.
Acknowledgment

The authors thank Weiyi Li, Jonathan Schantz and William McWilliams for their contributions to the animal studies.
REFERENCES


Legend for figures

Figure 1. Structures of vabicaserin and vabicaserin carbamoyl glucuronide (CG)

Figure 2. Summed mass chromatograms of plasma extracts from an individual human subject at 6 and 12 hr after administration of a single oral 500 mg dose of vabicaserin

Figure 3. Summed mass chromatograms of 0-4 and 4-12 hr urine samples from an individual human subject following administration of a single 50 mg oral dose of vabicaserin

Figure 4. Summed mass chromatogram of vabicaserin and its metabolites in monkey plasma

Figure 5. Incubations of \([^{14}\text{C}]\text{vabicaserin}\) (10 \(\mu\text{M}\)) with liver microsomes of mice, rats, dogs, monkeys and humans in the presence of UDPGA and NADPH at 37 °C for 20 minutes in a CO\(_2\)-enriched environment

Figure 6. Proposed fragmentation scheme and product ions of \(m/z\) 229 mass spectrum for vabicaserin

Figure 7. Proposed fragmentation scheme and product ions of \(m/z\) 449 mass spectrum for vabicaserin CG

Figure 8. Comparison of plasma CG/vabicaserin exposure ratios based on AUCs in various species.
Table 1. Characteristics of mouse, rat, dog, monkey and human liver microsomes utilized in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>Number of Subjects Pooled</th>
<th>P450 Content (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Male</td>
<td>20</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>18</td>
<td>0.54</td>
</tr>
<tr>
<td>Rat</td>
<td>Male</td>
<td>23</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>50</td>
<td>0.55</td>
</tr>
<tr>
<td>Dog</td>
<td>Male</td>
<td>5</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>0.43</td>
</tr>
<tr>
<td>Monkey</td>
<td>Male</td>
<td>10</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>1.31</td>
</tr>
<tr>
<td>Human</td>
<td>Male and female mixed</td>
<td>6</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Table 2. Concentrations (ng/mL) of vabicaserin and CG in plasma from healthy human subjects following a single vabicaserin dose \(^a\)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Time (hr)</th>
<th>Vabicaserin</th>
<th>CG</th>
<th>CG/vabicaserin</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>6</td>
<td>2.47 ± 2.21</td>
<td>114 ± 26.9</td>
<td>74.3 ± 59.6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.20 ± 1.14</td>
<td>12.4 ± 5.00</td>
<td>14.8 ± 7.87</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.40</td>
<td>3.4</td>
<td>10.8</td>
</tr>
<tr>
<td>AUC(_{0-24}) (^c)</td>
<td>28</td>
<td>816</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>

| 200      | 6         | 10.9 ± 7.71 | 352 ± 228 | 45.2 ± 34.0  |
|          | 12        | 5.60 ± 4.06 | 43.6 ± 17.1 | 14.3 ± 14.8  |
|          | 24        | 1.50 ± 1.15 | 7.67 ± 2.50 | 8.42 ± 6.68  |
| AUC\(_{0-24}\) \(^c\) | 125       | 2550        |        | 20             |

| 300      | 6         | 7.45 ± 7.48 | 237 ± 108 | 46.1 ± 21.6  |
|          | 12        | 4.23 ± 3.26 | 88.7 ± 51.8 | 23.5 ± 5.10  |
|          | 24        | 1.46 ± 0.43 | 28.9 ± 18.4 | 24.1 ± 22.7  |
| AUC\(_{0-24}\) \(^c\) | 91.5      | 2394        |        | 26             |

| 500      | 6         | 32.1 ± 12.2 | 1018 ± 444 | 35.4 ± 22.7  |
|          | 12        | 19.4 ± 6.56 | 178 ± 30.1 | 10.5 ± 5.92  |
|          | 24        | 5.45 ± 1.47 | 49.6 ± 32.6 | 10.7 ± 8.77  |
| AUC\(_{0-24}\) \(^c\) | 400       | 8008        |        | 20             |

\(^a\) Data are presented as mean ± S.D., N=3; \(^b\) Average of two subjects. \(^c\) AUC\(_{0-24}\) values were calculated with mean plasma concentrations using WinNonlin 5.1 and were presented as ng equivalents·hr/mL.
Table 3. CG-to-vabicaserin ratios in urine from healthy human subjects after a single vabicaserin dose

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Time (hr)</th>
<th>CG/vabicaserin</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0-4</td>
<td>476 ± 288</td>
</tr>
<tr>
<td>50</td>
<td>4-12</td>
<td>253 ± 117</td>
</tr>
<tr>
<td>50</td>
<td>12-24</td>
<td>111 ± 82.9</td>
</tr>
<tr>
<td>200</td>
<td>0-4</td>
<td>208 ± 33.9</td>
</tr>
<tr>
<td>200</td>
<td>4-12</td>
<td>292 ± 60.2</td>
</tr>
<tr>
<td>200</td>
<td>12-24</td>
<td>95.6 ± 129</td>
</tr>
<tr>
<td>300</td>
<td>0-4</td>
<td>537 ± 400</td>
</tr>
<tr>
<td>300</td>
<td>4-12</td>
<td>308 ± 222</td>
</tr>
<tr>
<td>300</td>
<td>12-24</td>
<td>198 ± 180</td>
</tr>
<tr>
<td>500</td>
<td>0-4</td>
<td>147 ± 85.9</td>
</tr>
<tr>
<td>500</td>
<td>4-12</td>
<td>100 ± 6.72</td>
</tr>
<tr>
<td>500</td>
<td>12-24</td>
<td>174 ± 124</td>
</tr>
</tbody>
</table>

a, Data are presented as mean ± S.D., N=3
Table 4. Plasma concentrations of total radioactivity, vabicaserin and CG (ng equivalents/mL) in mice, rats and dogs following a single oral administration of $[^{14}\text{C}]$vabicaserin

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Mouse (50 mg/kg, n=4-5)</th>
<th>Rat (5 mg/kg, n=3)</th>
<th>Dog (15 mg/kg, n=2-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Vabicaserin</td>
<td>CG</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3819 ± 622</td>
<td>582 ± 173</td>
<td>582 ± 199</td>
</tr>
<tr>
<td>4</td>
<td>3771 ± 512</td>
<td>475 ± 177</td>
<td>773 ± 293</td>
</tr>
<tr>
<td>8</td>
<td>3263 ± 624</td>
<td>385 ± 135</td>
<td>491 ± 116</td>
</tr>
<tr>
<td>24</td>
<td>597 ± 213</td>
<td>24 ± 16</td>
<td>213 ± 119</td>
</tr>
<tr>
<td>AUC_{0-24}^d</td>
<td>56357</td>
<td>6631</td>
<td>10097</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5250 ± 583</td>
<td>999 ± 181</td>
<td>344 ± 157</td>
</tr>
<tr>
<td>8</td>
<td>2868 ± 615</td>
<td>356 ± 135</td>
<td>375 ± 183</td>
</tr>
</tbody>
</table>

\(^a\) Data are presented as mean ± S.D. or the average of two subjects. \(^b\) ND, not detected. \(^c\) NA, data not available. \(^d\) AUC_{0-24} values were calculated with mean plasma concentrations using WinNonlin 5.1 and were presented as ng equivalents·hr/mL.
Table 5. Biliary excretion and percent distribution of vabicaserin and CG in rats following single oral administration of [14C]vabicaserin

<table>
<thead>
<tr>
<th>Sampling Period (hr)</th>
<th>Gender</th>
<th>Total 14C (%Dose)</th>
<th>Vabicaserin (%Biliary 14C)</th>
<th>CG (%Biliary 14C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>6.5 ± 3.4</td>
<td>1.7 ± 2.1</td>
<td>30 ± 8.6</td>
</tr>
<tr>
<td>0-4</td>
<td>Female</td>
<td>10 ± 3.7</td>
<td>1.1 ± 2.0</td>
<td>15 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>5.1 ± 2.2</td>
<td>0.4 ± 0.6</td>
<td>27 ± 19</td>
</tr>
<tr>
<td>4-8</td>
<td>Female</td>
<td>6.8 ± 0.8</td>
<td>1.5 ± 1.3</td>
<td>13 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>7.0 ± 1.1</td>
<td>2.5 ± 1.2</td>
<td>22 ± 18</td>
</tr>
<tr>
<td>8-24</td>
<td>Female</td>
<td>7.2 ± 3.4</td>
<td>2.8 ± 1.4</td>
<td>12 ± 3.4</td>
</tr>
</tbody>
</table>

a, Data are presented as mean S.D., n=3 or 4.
Table 6. Concentrations (ng/mL) of vabicaserin and CG in male monkey plasma following a single 25 mg/kg oral dose

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Time (hr)</th>
<th>Vabicaserin</th>
<th>CG</th>
<th>CG/vabicaserin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>20.8 ± 14.8</td>
<td>310 ± 300</td>
<td>17.5 ± 18.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12.2 ± 4.78</td>
<td>130 ± 138</td>
<td>9.55 ± 10.0</td>
</tr>
<tr>
<td>Plasma</td>
<td>8</td>
<td>10.9 ± 6.03</td>
<td>142 ± 222</td>
<td>9.33 ± 13.2</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>2.11 ± 0.74</td>
<td>4.05 ± 3.31</td>
<td>1.70 ± 1.18</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-24&lt;/sub&gt;</td>
<td>204</td>
<td>2473</td>
<td>12.1</td>
<td></td>
</tr>
</tbody>
</table>

a, Data are presented as mean ± S.D., N=3 or 4.

b, AUC<sub>0-24</sub> values were calculated with mean plasma concentrations using WinNonlin 5.1 and were presented as ng equivalents·hr/mL.
Vabicaserin
* Position of $^{14}$C label

Vabicaserin carbamoyl glucuronide (CG)
Control without microsomes

Mouse

Rat

Dog

Monkey

Human

-vabicaserin

-vabicaserin

-vabicaserin

-vabicaserin

-vabicaserin

-vabicaserin

CG

CG

CG

CG

Retention Time (min)