Assessment of the Mass Balance Recovery and Metabolite Profile of Avibactam in Humans and In Vitro Drug-Drug Interaction Potential

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Received October 29, 2013; accepted March 7, 2014

ABSTRACT

Avibactam, a novel non-β-lactam β-lactamase inhibitor with activity against Ambler class A, class C, and some class D enzymes is being evaluated in combination with various β-lactam antibiotics to treat serious bacterial infections. The in vivo mass balance recovery and metabolite profile of [14C] avibactam (500 mg/h infusion) was assessed in six healthy male subjects, and a series of in vitro experiments evaluated the metabolism and drug-drug interaction potential of avibactam. In the mass balance study, measurement of plasma avibactam (using a validated liquid chromatography-tandem mass spectrometry method) and total radioactivity in plasma, whole blood, urine, and feces (using liquid scintillation counting) indicated that most of the avibactam was excreted unchanged in urine within 12 hours, with recovery complete (>97% of the administered dose) within 96 hours. Geometric mean avibactam renal clearance (158 ml/min) was greater than the product of unbound fraction of drug and glomerular filtration rate (109.5 ml/min), suggesting that active tubular secretion accounted for some renal elimination. There was no evidence of metabolism in plasma and urine, with unchanged avibactam the major component in both matrices. Avibactam demonstrated in vitro substrate potential for organic anion transporters 1 and 3 (OAT1 and OAT3) proteins expressed in human embryonic kidney 293 cells (Km > 1000 μM; >10-fold the Cmax of a therapeutic dose), which could account for the active tubular secretion observed in vivo. Avibactam uptake by OAT1 and OAT3 was inhibited by probenecid, a potent OAT1/OAT3 inhibitor. Avibactam did not interact with various other membrane transport proteins or cytochrome P450 enzymes in vitro, suggesting it has limited propensity for drug-drug interactions involving cytochrome P450 enzymes.

Introduction

Infections caused by Gram-negative pathogens are becoming increasingly challenging to treat as a result of the global spread of multi-drug resistance among many clinically important species (Peleg and Hooper, 2010) and a limited armamentarium of new agents with intrinsic Gram-negative activity (Boucher et al., 2009). A key facet of bacterial drug resistance, particularly among the Enterobacteriaceae, is the expression of β-lactamases, a large family of hydrolytic enzymes that confer reduced susceptibility to β-lactam antibiotics (Kanj and Kanafani, 2011). Extended spectrum β-lactamases inactivate extended-spectrum cephalosporins, as well as penicillins, and typically require treatment with a carbapenem or β-lactam/β-lactamase inhibitor combination. Recently, the emergence of molecular class A β-lactamases with carbapenemase activity (e.g., Klebsiella pneumoniae carbapenemase [KPC]) has been reported; treatment options for such infections are severely limited (Hirsch and Tam, 2010; Nordmann et al., 2011). Avibactam, formerly known as NXL104, is a novel non-β-lactam inhibitor of Ambler class A and C (and some class D) β-lactamases, including KPC (Stachyra et al., 2009; Shlaes 2013; Zhanel et al., 2013) (Fig. 1). Avibactam has a unique mechanism of action involving covalent, slow, reversible β-lactamase inhibition (Ehmann et al., 2012), which restores the in vitro activity of β-lactam antibiotics, including ceftazidime, cefaroline, and aztreonam against extended-spectrum β-lactamases-producing pathogens (Livermore

http://dx.doi.org/10.1124/dmd.113.055335

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

ABBREVIATIONS: Ae, amount of total radioactivity or parent drug eliminated; AE, adverse event; AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; CYP450, cytochrome P450; ECG, electrocardiogram; HEK293, human embryonic kidney 293 cells; HPLC, high-performance liquid chromatography; KPC, Klebsiella pneumoniae carbapenemase; LC, liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry detection; LSC, liquid scintillation counting; MDCKII, Madin-Darby canine kidney II cells; MDR1, multidrug resistance 1 protein; MRP4, multidrug resistance-associated protein; OAT1/OAT3, organic anion transporters 1/3; OATP1B1/OATP1B3, organic anion transporting polypeptide proteins 1 and 3; OCT1/OCT2, organic cation transporters 1 and 2; PK, pharmacokinetic.

http://dmd.aspetjournals.org/content/suppl/2014/03/10/dmd.113.055335.DC1

Supplemental material to this article can be found at:
http://dx.doi.org/10.1124/dmd.113.055335

Drug Metab Dispos 42:932–942, May 2014

DRUG METABOLISM & DISPOSITION

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et al., 2011). Previous investigations have demonstrated that the pharmacokinetic (PK) profile of i.v. avibactam is dose-proportional up to 2000 mg (the highest dose tested), that elimination is largely by the renal route (Felices et al., 2010; Merdjan et al., 2007, 2010), and that there are no drug-drug interactions between avibactam and ceftazidime (Edeki et al., 2013). In these studies, the amount of unchanged avibactam eliminated in urine varied between 80% and 100%. To fully understand and characterize the elimination pathways of avibactam and to understand the impact of covalent, reversible tissue binding, a [14C] avibactam mass balance study was undertaken in healthy human subjects.

This report describes the results of the mass balance study as well as a series of additional in vitro studies undertaken to further characterize the disposition of avibactam, including assessments of the potential of avibactam as a substrate or inhibitor of human hepatic and renal transporter proteins and cytochrome P450 enzymes.

Materials and Methods

Overall Study Design and Objectives

The mass balance recovery study (ClinicalTrials.gov identifier NCT01448395) was an open-label, single-center phase I clinical trial conducted at Quotient Clinical, Nottingham, UK (with metabolite profiling of study samples conducted at AstraZeneca R&D, Waltham, MA). The study was performed in accordance with the Declaration of Helsinki and International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements, with protocol approval by an independent ethics committee; all participants provided written informed consent. The UK Department of Health Administration of Radioactive Substances Advisory Committee provided prior approval.

The primary objectives were to determine the mass balance and routes of [14C] avibactam metabolism and excretion after a single 500-mg i.v. dose of [14C] avibactam (this dose of avibactam is currently being investigated in combination with ceftazidime in phase III trials), to estimate the whole blood pharmacokinetic (PK) profile of i.v. avibactam is dose-proportional up to 2000 mg (the highest dose tested), that elimination is largely by the renal route (Felices et al., 2010; Merdjan et al., 2007, 2010), and that there are no drug-drug interactions between avibactam and ceftazidime (Edeki et al., 2013). In these studies, the amount of unchanged avibactam eliminated in urine varied between 80% and 100%. To fully understand and characterize the elimination pathways of avibactam and to understand the impact of covalent, reversible tissue binding, a [14C] avibactam mass balance study was undertaken in healthy human subjects.

This report describes the results of the mass balance study as well as a series of additional in vitro studies undertaken to further characterize the disposition of avibactam, including assessments of the potential of avibactam as a substrate or inhibitor of human hepatic and renal transporter proteins and cytochrome P450 enzymes.

Mass Balance Study

Subjects and Treatments. Healthy male subjects aged 30–65 years, with body mass index between 18 and 32 kg/m² and body weight between 50 and 100 kg, were eligible to participate; volunteers were required to have a clinically normal physical examination (including negative test results for drugs of abuse, alcohol, carbon monoxide breath test, hepatitis B surface antigen, antibodies to hepatitis C virus, and antibodies to HIV at the screening visit) and had to be willing to use an adequate method of contraception for 3 months from the day of dosing with the study drug. Exclusion criteria included clinically significant disease or clinically relevant abnormal findings in physical examination, vital signs, clinical chemistry, hematology, or urinalysis that, in the investigator’s opinion, could put the volunteer at risk by participation in the study; QT interval corrected for heart rate >450 ms or QT > 500 ms or other electrocardiogram (ECG) abnormality; and history of drug or alcohol abuse or smoking. After an overnight fast of ≥10 hours, subjects received a single clinically relevant dose of [14C] avibactam (Quotient Biosearch Ltd, Nottingham, UK) of approximately 500 mg diluted in 100 ml of 0.9% saline by 60 minutes of i.v. infusion. The target dose of 14C was to be no more than 300 μCi (11.1 MBq).

Assessments. Venous blood samples for analysis of whole blood and plasma radioactivity and plasma avibactam concentrations were collected via venipuncture/indwelling catheter at predose and at 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 hours postdose. Urine and feces samples for analysis of total radioactivity and avibactam were collected at predefined intervals (urine: –12–0 hours predose and 0–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, and 72–96 hours postdose; feces: –24–0 hours predose and 0–24, 24–48, 48–72, and 72–96 hours postdose). Subjects were discharged from the study unit at 96 hours, with scheduled assessments planned to continue to up to 168 hours (240 hours for feces) if required. Concentrations of avibactam in plasma and urine were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, for which the limits of quantification were 10.0 ng/ml (plasma) and 500 ng/ml (urine). Whole blood, plasma, and urine total radioactivity concentrations were measured using a liquid scintillation counting (LSC) method (feces samples were first combusted and the resultant CO2 collected into scintillation fluid before counting). The limits of detection for total radioactivity were 0.028 μg equiv/ml (plasma), 0.089 μg equiv/g (whole blood), 0.012 μg equiv/g (urine), and 0.07 μg equiv/g (feces).

Safety assessments included analysis of adverse events (AEs; coded using MedDRA version 14.1), laboratory assessments of blood and urine, vital signs, ECG, and physical examinations.

Metabolite Profiling. Plasma samples obtained during the first 4 hours postdose were pooled across subjects in a time-interval proportional fashion (Hamilton et al., 1981) such that the final pool represented the area under the curve (AUC) over the 4-hour postdose period. Pooled plasma was denatured and precipitated with a 2-fold excess volume of acetonitrile to extract drug-related material. The supernatant obtained after centrifugation was evaporated and reconstituted in water before analysis. Similarly, urine samples obtained during the 0–24 hour collection interval were pooled across subjects. The volume of a given sample represented in the pool was weighted based on the net weight of urine collected for the time interval. The pooled urine was analyzed without further processing.

LC-MS using LTQ-Orbitrap was used to analyze avibactam and its related components in pooled plasma and urine samples (see Supplemental Methods).
In Vitro Studies

Membrane Transporter Study. Assay systems to evaluate the inhibition and substrate (uptake) potential of avibactam for human membrane transporters were performed using transport protein cDNA stably transfected into Madin-Darby canine kidney II (MDCKII) cells, human embryonic kidney (HEK293) cells, or human membrane vesicles expressing native transporter. Avibactam substrate and inhibition potential for breast cancer resistance protein (BCRP) and multidrug resistance 1 protein (MDR1) were assessed with transporters expressed in MDCKII cells. Human multidrug resistance-associated protein (MRP4), organic anion transporting polypeptide proteins 1 and 3 (OATP1B1 and OATP1B3), organic anion transporters 1 and 3 (OAT1 and OAT3), and organic cation transporters 1 and 2 (OCT1 and OCT2) expressed in HEK293 cells were assessed for avibactam inhibition potential. In addition, MRP4, OAT1, OAT3, and OCT2 expressed in HEK293 cells were assessed for avibactam substrate potential. Inhibition potential of avibactam for human bile salt export pump (BSEP) was assessed in membrane vesicles isolated from cells overexpressing BSEP and control membrane vesicles (see Supplemental Methods). Inhibition potential of avibactam was assessed by measurement of radiolabeled model substrate uptake in the presence of avibactam (AstraZeneca, Macclesfield, UK). Substrate potential of avibactam was assessed by measuring the bidirectional transport (MDCKII cells) or uptake (HEK293 cells) of [14C] avibactam in the absence or presence of the following model inhibitors: MDR1, ketocozolone (Sigma-Aldrich, Zwijndrecht, Netherlands); BCRP, Ko143 (Allen et al., 2002; synthesized by Prof. G. J. Koomen, Van’t Hoff Institute for Molecular Sciences, Netherlands/Tocris Bioscience, Bristol, UK); MRPS, dipridamole; OAT1 and OAT3, probenecid; OCT2, quinidine (all from Sigma-Aldrich). Empty vectors treated with test substances were used as negative controls. Effects of avibactam at concentrations of 0.5–300 μM on cytotoxicity or monolayer cell viability were assessed for the MDCKII and HEK293 control cells using a neutral red uptake assay (Borenfreund and Puerner, 1985) with Triton-X100 (1%) as positive control.

Cytocrome P450 Induction Study. The in vitro induction potential of avibactam to inhibit the activity of human cytochrome P450 isozymes 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4/5 was investigated in human liver microsomes (see Supplemental Table 1 and Supplemental Methods). In one study, microsomes (individual and pooled samples from 15–30 donors) were preincubated with avibactam with and without NADPH (2 mM) for 20 minutes to evaluate time-dependent inhibition of cytochrome enzymes. Parallel incubations were done with reference inhibitors as positive controls. Since a minor inhibition of CYP2C9 was observed in the first study, a further study was conducted using a similar method to evaluate the potential of avibactam to inhibit CYP2C9 activity at concentrations up to 5000 μM in pooled microsomes from 50 donors with preincubation with NADPH (2 mM) for 10 minutes. Enzymatic activities were determined by measuring the concentration of formed metabolite using high-performance liquid chromatography (HPLC) with ultraviolet detection method for all enzymes except CYP2A6, which was determined by fluoromometry.

**Cytochrome P450 Inhibition Studies.** The in vitro potential of avibactam to inhibit the activity of human cytochrome P450 isozymes 1A2, 2B6, 2C9, 2E1, and 3A4 was investigated in freshly isolated human hepatocytes from three donors (see Supplemental Table 2 and Supplemental Methods). The induction potential of avibactam was tested at 200–5000 μM.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Key pharmacokinetic (PK) parameters for total radioactivity and avibactam after a single intravenous infusion of [14C] avibactam (PK population; n = 6)</td>
</tr>
<tr>
<td>Terminal slopes (λi) for radioactivity in whole blood could not be reliably defined for any subject and could be defined for only one subject for plasma radioactivity but were reliably defined for plasma avibactam; hence, all λi-dependent parameters (λi, θi, AUCi, CLi, Vss, and CLi) were estimated only for plasma avibactam.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Radioactivity in Plasma</th>
<th>Total Radioactivity in Whole Blood</th>
<th>Avibactam in Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/mL)</td>
<td>22.4 (17.4)</td>
<td>14.6 (15.2)</td>
<td>23.1 (11.1)</td>
</tr>
<tr>
<td>Cmax (h)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
</tr>
<tr>
<td>AUCo–c (μg.h/mL)</td>
<td>47.6 (22.7)</td>
<td>36.2 (24.0)</td>
<td>47.10 (20.0)</td>
</tr>
<tr>
<td>AUCo–c (μg.h/mL)b</td>
<td>NC</td>
<td>NC</td>
<td>47.20 (20.0)</td>
</tr>
<tr>
<td>θ1/2 (h)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>NC</td>
<td>NC</td>
<td>2.778 (0.6)</td>
</tr>
<tr>
<td>Vss (ml)</td>
<td>NC</td>
<td>NC</td>
<td>1.887 (16.0)</td>
</tr>
<tr>
<td>CLi (ml/min)</td>
<td>NC</td>
<td>NC</td>
<td>21.200 (10.7)</td>
</tr>
<tr>
<td>CLR (ml/min)</td>
<td>NC</td>
<td>NC</td>
<td>158.0 (21.2)</td>
</tr>
<tr>
<td>Fe 0–96 h (%)</td>
<td>97.0 (1.0)</td>
<td>0.2 (0.03)</td>
<td>84.9 (11.8)</td>
</tr>
</tbody>
</table>

AUCo–c: area under the concentration-time curve from dosing extrapolated to infinity; AUCo–c: area under the concentration-time curve from dosing to the last measurable time point; Cmax: maximum (peak) concentration; Fe: amount of total radioactivity or parent drug eliminated expressed as a percentage of the dose administered; MRT: renal clearance; CLi: total clearance; Cmax: maximum (peak) concentration; Fe: amount of total radioactivity or parent drug eliminated expressed as a percentage of the dose administered; MRT: mean residence time; NC, not calculated; θ1/2, terminal half-life; tmax, time to reach Cmax; Vss, volume of distribution at steady state.

*Values shown are geometric mean (%CV) except for tmax, which are median (range), and t1/2 and Fe%, which are arithmetic mean (S.D.)

*Units are μg equiv/ml for total radioactivity in plasma and μg equiv mL for total radioactivity in whole blood.

*Units are μg equiv/ml for total radioactivity in plasma and μg equiv mL for total radioactivity in whole blood.

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expected to cover therapeutic concentrations and span a safety margin >50-fold the $C_{\text{max}}$ of a 500-mg dose. Hepatocytes were incubated in 0.5 ml of hepatocyte incubation medium at 37 ± 2°C for 72 ± 4 hours (induction phase) with avibactam, a fixed concentration of reference inducer (positive controls), or hepatocyte incubation medium only (negative controls). After completion of the induction phase, supernatants were removed and hepatocytes were incubated in Krebs’ Henseleit buffer containing 3 mM salicylamide and marker substrate for 3 hours ± 15 minutes (reaction phase). Proteins were then precipitated in acetonitrile, and substrate metabolites (markers of enzyme activity) were analyzed by assay-specific HPLC and fluorimetry methods.

**Fig. 2.** Geometric mean concentrations of avibactam in plasma (determined using LC-MS/MS detection) and total $^{14}$C radioactivity in plasma and whole blood after a single i.v. infusion of 500 mg of $^{[14C]}$ avibactam in healthy male subjects (PK population; $n = 6$). Error bars represent the interval of geometric mean/geometric S.D., geometric mean × geometric mean S.D.

**Fig. 3.** Mean cumulative recovery of avibactam and total radioactivity in urine as a percentage of the dose administered (Fe%) after a single i.v. infusion of 500 mg of $^{[14C]}$ avibactam in healthy male subjects (PK population; $n = 6$). Error bars represent S.D.

**Fig. 4.** Representative LC radiochromatograms (A) 0- to 4-hour pooled human plasma after a single i.v. infusion of 500 mg of $^{[14C]}$ avibactam in healthy male subjects (PK population; $n = 6$) and (B) control human plasma fortified with $^{[14C]}$ avibactam.
Microsomal Metabolism Study. The in vitro metabolism of avibactam was evaluated in pooled microsomes (all supplied by Biopredic, Saint Grégoire, France) isolated from liver samples from humans \((n = 15)\), CD1 mice \((n = 9)\), Sprague-Dawley rats \((n = 10)\), New Zealand rabbits \((n = 10)\), and beagle dogs \((n = 5)\). Microsomes were incubated with avibactam and relevant cofactors at nominal concentrations of 3.125 and 200 \(\mu M\) for up to 90 minutes (each experiment was performed in duplicate). At the end of the incubation, reactions were terminated by the addition of 0.5 ml of ice-cold acetonitrile and analyzed.

![Fig. 5. Representative LC radiochromatograms (A) 0- to 24-hour pooled urine after a single i.v. infusion of 500 mg of \(^{14}C\) avibactam in healthy male subjects (PK population; \(n = 6\)) and (B) control \(^{14}C\) avibactam dose solution.](image)

**TABLE 2**

Summary of results from the in vitro evaluation of avibactam as a substrate for membrane transporter proteins

<table>
<thead>
<tr>
<th>Transporter Assay</th>
<th>Test System</th>
<th>[^{14}C]-Avibactam Concentration</th>
<th>Transport Ratio (a)</th>
<th>Reference Inhibitor</th>
<th>Inhibition (b)</th>
<th>Substrate Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\mu M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>MDCKII-MDR1 cells</td>
<td>0.58</td>
<td>1.39</td>
<td>Ketoconazole 25 ((\mu M))</td>
<td>17.3</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDCKII-BCRP cells</td>
<td>0.58</td>
<td>1.35</td>
<td>Ko143 (1 (\mu M))</td>
<td>35.6</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.16</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.5</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td>HEK-MRP4 cells</td>
<td>1.23</td>
<td>0.81</td>
<td>Dipyridamole (100 (\mu M))</td>
<td>NC</td>
<td>No(^c)</td>
</tr>
<tr>
<td></td>
<td>HEK-OAT1 cells</td>
<td>1.38</td>
<td>6.45</td>
<td>Probenecid (100 (\mu M))</td>
<td>70.4</td>
<td>Yes ((K_m &gt; 1000 \mu M))</td>
</tr>
<tr>
<td></td>
<td>HEK-OAT3 cells</td>
<td>1.38</td>
<td>4.34</td>
<td>Probenecid (100 (\mu M))</td>
<td>56.1</td>
<td>Yes ((K_m &gt; 1000 \mu M))</td>
</tr>
<tr>
<td></td>
<td>HEK-OCT2 cells</td>
<td>1.38</td>
<td>0.88</td>
<td>Quinidine (100 (\mu M))</td>
<td>NI</td>
<td>No</td>
</tr>
</tbody>
</table>

**Notes:**

- BCRP, breast cancer resistance protein; HEK, human embryonic kidney; MDCKII, Madin-Darby canine kidney II; MDR1, multidrug resistance 1 protein; MRP4, multidrug resistance-associated protein 4; OAT1 and OAT3 organic anion transporters 1 and 3; OCT2, organic cation transporter 2; NC, not calculated; NI, no significant inhibition observed \((<10\%)\).
- \(^a\)MDCKII cells: Corrected efflux ratio = mean \(P_{app} \ b - a \ b\) from MDCKII-MDR1 or MDCKII-BCRP/mean \(P_{app} \ a - a \ b\) from MDCKII-control; HEK cells: Uptake ratio = \(mean (P_{app} \ b-a \ b) / mean (P_{app} \ a-a \ b)\) from MDCKII-control.
- \(^b\)MDCKII cells: Inhibition \(= 100 - \{(mean (P_{app} \ b-a \ (+inhibitor))/mean (P_{app} \ b-a \ (no inhibitor)))\} \times 100\); HEK cells: % inhibition = 100 – \((\text{mean uptake (+ inhibitor)/mean uptake (- inhibitor)}) \times 100\%\).
- \(^c\)As the inhibitor had no effect (increase) on the accumulation of \(^{14}C\) avibactam, it was concluded that avibactam is not a potential substrate for MRP4 transporter proteins when expressed in HEK cells.
Subjects and completed the study between October 17 and November 11, 2011. Five subjects were white, and one subject was English-Caribbean.

**Mass Balance Study**

**Subject Characteristics.** Six healthy male subjects were enrolled and completed the study between October 17 and November 11, 2011. Subjects’ age ranged from 36 to 63 years (mean ± S.D. 48.0 ± 10.6 years). Five subjects were white, and one subject was English-Caribbean. Mean ± S.D. body mass index was 25.82 ± 2.82 kg/m². The mean ± S.D. creatinine clearance was 119 ± 20 ml/min. All six subjects received a single dose of 500 mg of [14C] avibactam and were included in the PK and safety analysis populations.

**Pharmacokinetic and Total Radioactivity Assessments.** Key PK parameters for avibactam in plasma and total radioactivity in plasma and whole blood are shown in Table 1. Plasma avibactam concentrations peaked at the end of the infusion and declined in a biphasic manner thereafter (Fig. 2). Quantifiable concentrations of avibactam in plasma were recorded for up to 16 to 24 hours after the start of the infusion. Similarly, maximum concentrations of radioactivity in plasma and whole blood were observed at 1 hour after the start of infusion (i.e., at the end of the infusion) and declined in a biphasic manner after the end of the infusion (Fig. 2). Most of the total radioactivity in plasma was gone by 12 hours, but a small fraction of total radioactivity remained detectable for 24–72 hours after start of the infusion.

Geometric mean avibactam and total radioactivity concentrations in plasma were similar up to 8 hours after start of the infusion. From 12 hours after the start of the infusion until concentrations approached the lower limits of quantification, concentrations of avibactam in plasma appeared to decline more quickly than those for total radioactivity (Fig. 2). Geometric mean plasma and whole blood concentrations of total radioactivity were similar up to 8 hours after start of the infusion (the geometric mean ratio was 1.204 at 6 hours), although after 8 hours, the ratio decreased (geometric mean ratio was 0.317 at 24 hours after start of the infusion), suggesting that there was limited binding of total radioactivity to the cellular components of whole blood during the first 8 hours. Since the total radioactivity measurements at later time points were near the limits of quantification, terminal PK parameters could not be reliably estimated.

Based on LC-MS/MS quantitation using the validated bioanalytical method, a mean of 84.9% (range, 66.6%–101.3%) of unchanged avibactam was recovered from urine within 96 hours, with 40% to 70% recovered within the first 2 hours after start of the infusion. The geometric mean avibactam renal clearance rate was 84.6% of the total clearance rate, consistent with the predominantly renal route of avibactam excretion (Table 1).

Cumulative recovery (Fe%) of avibactam and total radioactivity from urine from −12 hour predose to 96 hours postdose are shown in Fig. 3. A mean of 97.2% (range, 95.6%–98.3%) of administered radioactivity was recovered within 96 hours: 97.0% (range, 95.3%–98.1%) from urine; 0.2% (range, 0.17%–0.23%) from feces (Table 1). Subjects were discharged from the clinical unit at 96 hours postdose as mass balance recovery was complete. Most of the administered radioactivity (mean, 95.6%) was recovered from urine within 12 hours of dosing, with 62.4% recovered in the first 2 hours (Fig. 3).

**Safety.** A single AE of headache occurring approximately 4 days after discharge from the clinic (reported as mild and considered unrelated to study treatment) was reported by one subject. No other AEs were experienced by any other subject, and there were no discontinuations because of AEs. Mean hematology and clinical chemistry values were within normal reference ranges at admission and discharge, and there were no clinically significant changes from baseline for any parameter. There were no notable mean changes from baseline for any vital signs or ECG parameters or abnormal physical examination findings postdose that were not present at screening.

**Metabolite Profiling.** The recovery of radioactivity after extraction, removal, and reconstitution of the pooled human plasma sample was 98.6%. The recoveries of radioactivity from the LC column during a gradient run, determined from an injection of processed pooled plasma or urine were 88% and 93%, respectively. Representative LC chromatograms for pooled plasma and urine are shown in Figs. 4 and 5, respectively.

Parent avibactam and one related product (an uncharacterized degradant) were observed in the pooled plasma sample, with parent avibactam accounting for 72.5% of total radioactivity and the unknown degradant accounting for 27.5% (Fig. 3). This unknown peak was also observed in

<table>
<thead>
<tr>
<th>Transporter Assay</th>
<th>Test System</th>
<th>Reference Substrate</th>
<th>Avibactam Concentration</th>
<th>Inhibition at Maximum Concentration&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Inhibition Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR1</td>
<td>MDCKII-MDR1 cells</td>
<td>[3H]-digoxin (0.05 µM)</td>
<td>100</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>BCRP</td>
<td>MDCKII-BCRP cells</td>
<td>[14C]-PhIP (1 µM)</td>
<td>100</td>
<td>14.4</td>
<td>No</td>
</tr>
<tr>
<td>MRPI</td>
<td>HEK-MRP4 cells</td>
<td>[4C]-bis-POM-PMEA (1 µM)</td>
<td>100</td>
<td>NC</td>
<td>No&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>HEK-OATP1B1 cells</td>
<td>[3H]-estradiol-17β-D-glucuronide (1 µM)</td>
<td>100, 500, 1000</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>HEK-OATP1B3 cells</td>
<td>[3H]-estradiol-17β-D-glucuronide (1 µM)</td>
<td>100, 500, 1000</td>
<td>NI</td>
<td>No</td>
</tr>
<tr>
<td>OAT1</td>
<td>HEK-OAT1 cells</td>
<td>[3H]-para-α-mono-hypuric acid (2 µM)</td>
<td>100, 500, 1000</td>
<td>32.5</td>
<td>Yes (IC&lt;sub&gt;50&lt;/sub&gt; &gt; 1000 µM)</td>
</tr>
<tr>
<td>OAT3</td>
<td>HEK-OAT3 cells</td>
<td>[3H]-estrone-3-sulfate (2 µM)</td>
<td>10–1000</td>
<td>46.7–53.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Yes (IC&lt;sub&gt;50&lt;/sub&gt; &gt; 1000 µM)</td>
</tr>
<tr>
<td>OCT2</td>
<td>HEK-OCT2 cells</td>
<td>[3H]-tetraethyl ammonium (10 µM)</td>
<td>100, 500, 1000</td>
<td>16.0</td>
<td>No</td>
</tr>
<tr>
<td>OCT2</td>
<td>HEK-OCT2 cells</td>
<td>[3H]-meflozin (10 µM)</td>
<td>100, 500, 1000</td>
<td>16.0</td>
<td>No</td>
</tr>
<tr>
<td>BSEP</td>
<td>Human membrane vesicles</td>
<td>[3H]-taurocholate (2 µM)</td>
<td>1–1000</td>
<td>NI</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup>IC<sub>50</sub> = Mean ATP-dependent uptake (+ inhibitor)/Mean ATP-dependent uptake (− inhibitor) *100%

<sup>b</sup>MDCKII cells: % inhibition = 100 – ((mean (P<sub>app</sub> b = a (inhibitor))/mean (P<sub>app</sub> b = a (no inhibitor)))*100%; HEK cells: % inhibition = 100 – (mean uptake (+ inhibitor)/mean uptake (− inhibitor))*100%; membrane vesicles: % inhibition = 100 – (mean ATP-dependent uptake (+ inhibitor)/mean ATP-dependent uptake (− inhibitor))*100%.

<sup>c</sup>Because of the method that could not be used due to the accumulation of [14C]-bis-POM-PMEA, it was concluded that avibactam did not inhibit MRPI transporter proteins in this assay.

<sup>d</sup>Data from two independent experiments.
control human plasma when fortified with [14C] avibactam undergoing the sample processing steps. As a result of the very low signal-to-noise ratio on the plasma radiochromatogram (Fig. 4), significant sample concentration procedures were undertaken but proved unsuccessful.

Parent avibactam and decarboxylated avibactam (metabolite M1) were observed in pooled urine. Parent avibactam was the major component, accounting for 93.0% of the radioactivity, and M1 accounting for 7.0% (Fig. 5). A small amount of M1 was also observed in the dose solution, indicating potential degradation either on storage or through sample processing. This metabolite was also previously observed in the rat and dog metabolism studies (data not shown). The full-scan MS and MS/MS and fragmentation details of avibactam and M1 are shown in Supplemental Fig. 1 and Supplemental Fig. 2, respectively.

In Vitro Studies

Membrane Transporter Study. Results of the transporter substrate and inhibition studies are shown in Tables 3 and 4. Avibactam was not a substrate of MDR1 or BCRP expressed in MDCKII cells (Table 2) and did not demonstrate inhibitory activity in these assay systems (Table 3). Similarly, avibactam did not inhibit MRP4 or exhibit substrate or inhibitory activity for human OCT2 proteins expressed in HEK293 cells.

Avibactam did show potential substrate activity for the human OAT1 and OAT3 proteins expressed in the HEK293 cell system ($K_m > 100 \mu M$, equivalent to 265.2 $\mu g/ml$). The reference OAT1/OAT3 inhibitor probenecid (100 $\mu M$) inhibited the uptake of avibactam by 70.4% in HEK293-OAT1 cells and by 56.1% in HEK293-OAT3 cells (Table 2). In addition, avibactam demonstrated the potential to inhibit human OAT1 and OAT3 transporters in the HEK293 cell system ($IC_{50}$ values > 1000 $\mu M$; Table 3), inhibiting uptake of the reference substrate by 32.5% (OAT1) and 46.7%–53.2% (OAT3) at 1000 $\mu M$. Avibactam at concentrations up to 1000 $\mu M$ did not inhibit OATP1B1, OATP1B3, or OCT1 in the HEK293 cell system or the BSEP transporter protein in the vesicle system (Table 3).

Cytochrome P450 Inhibition Studies. Incubation of microscope preparations with reference inhibitors demonstrated the viability of the assay systems. In the initial study, nominal avibactam concentrations up to 200 $\mu M$ showed minimal or no inhibition of all the cytochrome enzymes evaluated except CYP2C9 (Table 4). For CYP2C9, a mean of 29% inhibition versus controls was observed at 200 $\mu M$ avibactam (equivalent to 53.0 $\mu g/ml$). To fully characterize this inhibition, a higher concentration range of avibactam was evaluated in a further study using microsomes from a different set of sample donors. A mean of 36% inhibition of CYP2C9 was observed at 5000 $\mu M$ avibactam (the highest concentration tested), and no inhibition was observed at lower avibactam concentrations. In both studies, at the lower avibactam concentrations assessed (100–1000 $\mu M$), CYP2C9 inhibition was ≤10% and not concentration dependent. The reason for the apparent inhibition of CYP2C9 in the in vitro studies is unknown. Since the plasma $C_{max}$ of a therapeutic dose of avibactam is expected to be 26 $\mu g/ml$, the potential for clinically relevant CYP2C9 interactions is considered unlikely.

Cytochrome P450 Induction Study. Avibactam showed no induction potential on the activity of CYP1A2, CYP2B6, CYP2C9, and CYP3A4 in human hepatocytes over a concentration range of 200–5000 $\mu M$ (Table 5). A slight induction of CYP2E1 was observed in two of three donors (1.4- and 1.5-fold) at 5000 $\mu M$ (not considered clinically relevant). Positive controls demonstrated a more than 2-fold induction of the cytochrome isomers evaluated (1.5-fold for CYP2E1 in two of three donors), confirming the functionality of the assay systems used.
Microsomal Metabolism Study. The positive control agents used in the study were metabolized to their respective metabolites, indicating the viability of the microsomes. Results of the incubations with avibactam are shown Fig. 6. In mouse, rabbit, dog, and human microsomes, no avibactam metabolism was observed, and no metabolic products were detected at either 3.125 or 200 μM. However, in rabbit and rat microsomes at 200 μM, a mean of 12% to 13% depletion of the initial avibactam concentrations at time 0 was seen after incubation for 90 minutes, but no metabolites were detected.

Discussion

The studies presented here provide additional information on the disposition of avibactam in healthy volunteers and highlight the limited propensity for avibactam to elicit drug-drug interactions through assessment in vitro interaction with human membrane transporters and hepatic cytochrome enzymes. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previous reports of avibactam metabolism by cytochrome isoforms. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previous reports of avibactam metabolism by cytochrome isoforms. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previous reports of avibactam metabolism by cytochrome isoforms. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previous reports of avibactam metabolism by cytochrome isoforms. In the mass balance study, after a 1-hour i.v. infusion of 500 mg of [14C] avibactam, a mean of >93% of the administered dose was recovered in urine within 12 hours; >60% was recovered in the first 2 hours. This finding is consistent with previous reports of avibactam metabolism by cytochrome isoforms.

In vitro evaluation of avibactam induction potential of human cytochrome P450 enzymes

The results of individual donors (D1–D4) are shown as fold induction of cytochrome activity.

<table>
<thead>
<tr>
<th>Donor</th>
<th>CYP1A2</th>
<th>CYP2B6</th>
<th>CYP2C9</th>
<th>CYP3A4</th>
<th>CYP2E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>14.8</td>
<td>11.9</td>
<td>27.3</td>
<td>24.4</td>
<td>9.5</td>
</tr>
<tr>
<td>D2</td>
<td>4.4</td>
<td>9.5</td>
<td>19.3</td>
<td>10.7</td>
<td>0.5</td>
</tr>
<tr>
<td>D3</td>
<td>1.3</td>
<td>1.0</td>
<td>1.3</td>
<td>0.9</td>
<td>1.5</td>
</tr>
<tr>
<td>D4</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
<td>0.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*In incubations with hepatocytes from donor 3 were not evaluated because the plated hepatocytes lacked the confluence to produce a reliable turnover of the marker substrate; hepatocytes from a fourth donor were assessed instead.
filtration rate for unbound drug \((0.92 \times 119 = 109.5 \text{ ml/min})\), suggestive of active tubular secretion of avibactam in addition to glomerular filtration. In the in vitro membrane transporter study, avibactam was both a substrate and potential inhibitor of human OAT1 and OAT3 transporters \((K_m \text{ and } IC_{50} > 1000 \ \mu M, \text{ equivalent to } 265.2 \ \mu g/ml)\), suggesting that these transporters might be responsible for the active secretion of avibactam observed in vivo. Probenecid (a potent OAT inhibitor), at a concentration of \(100 \ \mu M\), inhibited the uptake of avibactam by OAT1 and OAT3 in the HEK293 assay system.

OAT1 and OAT3 transporters are expressed on the basolateral membrane of the renal proximal tubule and are considered the rate-limiting step in the renal clearance of organic anion drugs, metabolites, and toxins in vivo (Gallegos et al., 2012). In addition to their role in renal drug clearance, both these transporters have potential roles in clinical drug-drug interactions (Giacomini et al., 2010). Avibactam IC_{50} values were more than 20-fold higher than the mean \(C_{max}\) of a 500-mg dose \((23.1 \ \mu g/ml)\), and with the rapid elimination of avibactam, the potential for drug-drug interactions involving other substrates of OAT1 and OAT3 is thought to be low. Nonetheless, potent inhibitors of OAT1 and OAT3 (such as probenecid) have the potential to alter the elimination of avibactam when coadministered. Since a clinical interaction study of avibactam and probenecid has not been conducted, coadministration of avibactam with probenecid is not recommended.

Avibactam had no significant effects on the other human renal and hepatic transporters evaluated (BRCP, MDRP, OCT2, OATP1B1, OATP1B3 and BSEP). Hence, drug-drug interactions involving codosing of avibactam with substrates or inhibitors of these transporters are considered unlikely.

At avibactam concentrations up to \(200 \ \mu M\) \((53.1 \ \mu g/ml\) or approximately 2-fold the mean \(C_{max}\) of a 500-mg dose\), inhibition of human CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5 was mostly absent or negligible and \(\leq 50\%\) in all cases, indicating IC_{50} values \(>200 \ \mu M\) for all cytochrome P450 enzymes assessed. A 29\% inhibition of CYP2C9 activity was observed at \(200 \ \mu M\) avibactam, and
36% inhibition was observed at 5000 μM. However, inhibition of CYP2C9 by avibactam was determined to be unspecific and negligible at concentrations ≤1000 μM (265.2 μg/ml), which greatly exceed any clinically relevant exposure. Avibactam showed no significant induction of cytochrome enzymes in freshly isolated hepatocytes at therapeutic concentrations. However, a slight induction of CYP2E1 was observed in two of three donors at the highest concentration tested (5000 μM; equivalent to 1326 μg/ml or >50-fold the plasma Cmax of a 500-mg dose). Hence, the potential for cytochrome P450 mediated drug-drug interactions with coadministering avibactam is unlikely. In summary, as well as supporting previous findings indicating that avibactam is predominantly eliminated unchanged by the renal route, these data suggest there is a limited role of OAT1 and OAT3 renal drug transporters in avibactam secretion and also highlight the low likelihood of interaction with a range of cytochrome enzymes. Active secretion via OAT1 and OAT3 transporters may account for a small proportion of overall avibactam clearance (indicating a potential for clinical drug-drug interactions when coadministering avibactam with other compounds that inhibit OAT1 and/or OAT3). Extensive in vitro investigations found no evidence of potential for avibactam drug-drug interactions involving other renal or hepatic transporters or hepatic cytochrome P450 enzymes.

Acknowledgments
Medical writing support was provided by Mark Waterlow of Prime Medica Ltd, Knutsford, Cheshire, UK, funded by AstraZeneca. The design and conduct of the studies, as well as analysis of the study data and opinions, conclusions, and interpretation of the data, are the responsibility of the authors. The authors thank Fanny Boisivé and Béatrice Lopez (Biopredic, France), Friedrich Krätzer and Katharina Fuchs (GenPharmTox BioTech AG, Germany), Paul Newell (AstraZeneca, UK), and Rianne AF de Ligt (Netherlands Organisation for Applied Scientific Research, Netherlands) for their involvement in these studies.

Authorship Contributions
Participated in research design: Vishwanathan, Mair, Gupta, Clarkson-Jones.
Conducted experiments: Vishwanathan, Mair, Atherton.

Contributed new reagents or analytic tools: Vishwanathan.

Performed data analysis: Vishwanathan, Gupta, Atherton, Clarkson-Jones.
Wrote or contributed to the writing of the manuscript: Vishwanathan, Mair, Gupta, Atherton, Clarkson-Jones, Edeki, Das.

References
Edeki T, Armstrong J, and Li J. Pharmacokinetics of avibactam (AVI) and ceftazidime (CAZ) following separate or combined administration in healthy volunteers. Poster A-1019 presented at 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 10–13 September, 2013, Denver, CO.

Avibactam Mass Balance and Drug-Drug Interaction Studies 941

Fig. 7. Proposed metabolic pathway for avibactam in humans.

Contributed new reagents or analytic tools: Vishwanathan.

Performed data analysis: Vishwanathan, Gupta, Atherton, Clarkson-Jones.
Wrote or contributed to the writing of the manuscript: Vishwanathan, Mair, Gupta, Atherton, Clarkson-Jones, Edeki, Das.

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Avibactam Mass Balance and Drug-Drug Interaction Studies 941

Fig. 7. Proposed metabolic pathway for avibactam in humans.


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