Investigating the Enteroenteric Recirculation of Apixaban, a Factor Xa Inhibitor: Administration of Activated Charcoal to Bile Duct-Cannulated Rats and Dogs Receiving an Intravenous Dose and Use of Drug Transporter Knockout Rats

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ABSTRACT

The study described herein investigated the impact of intestinal excretion (IE; excretion of drug directly from circulation to intestinal lumen), enteroenteric recirculation (EER), and renal tubule recirculation (RTR) on apixaban pharmacokinetics and disposition. The experimental approaches involve integrating apixaban elimination pathways with pharmacokinetic profiles obtained from bile duct-cannulated (BDC) rats and dogs receiving i.v. doses together with oral administration of activated charcoal (AC). Additionally, the role of P-gp (P-glycoprotein; abcb1) and BCRP (breast cancer resistance protein; abcg2) in apixaban disposition was evaluated in experiments using transporter inhibitors and transporter knockout (KO) rats. Approximately 20–50% of an apixaban i.v. dose was found in feces of BDC rats and dogs, suggesting IE leading to fecal elimination and intestinal clearance (IC). The fecal elimination, IC, and systemic clearance of apixaban were increased upon AC administration in both BDC rats and dogs and were decreased in BDC rats dosed with GF-120918, a dual BCRP and P-gp inhibitor. BCRP appeared to play a more important role for absorption and intestinal and renal elimination of apixaban than P-gp in transporter-KO rats after oral i.v. dosing, which led to a higher level of active renal excretion in rat than other species. These data demonstrate that apixaban undergoes IE, EER, and RTR that are facilitated by efflux transporters. Intestinal reabsorption of apixaban could be interrupted by AC even at 3 hours post-drug dose in dogs (late charcoal effect). This study demonstrates that the intestine is an organ for direct clearance and redistribution of apixaban. The IE, EER, and RTR contribute to overall pharmacokinetic profiles of apixaban. IE as a clearance pathway, balanced with metabolism and renal excretion, helps decrease the impacts of intrinsic (renal or hepatic impairment) and extrinsic (drug-drug interactions) factors on apixaban disposition.

Introduction

Apixaban (Fig. 1), 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide, a highly selective, oral, direct inhibitor of Factor Xa, a protease enzyme that plays a pivotal role in the coagulation cascade, is a newly approved anticoagulant (Lassen et al., 2007; Connolly et al., 2011; Granger et al., 2011). Following oral administration of [14C]apixaban in humans, both metabolism (25%) and urinary excretion (27%) were important clearance pathways (Raghavan et al., 2009; Zhang et al., 2009a). Approximately 20–50% of radioactive dose was recovered in feces as the parent from rats, dogs, and humans following oral administration of [14C]apixaban. Plasma radioactivity profiling has shown that apixaban represents >90% of all circulating drug-related components in rats and dogs and 75% in humans (Raghavan et al., 2009; Wang et al., 2011; Zhang et al., 2009a). Apixaban is metabolically stable in incubations with liver microsomes and hepatocytes and has a relatively low clearance with total plasma clearance of <5% hepatic blood flow in rats, dogs, and humans (He et al., 2011; Wong et al., 2011). Apixaban has a fair water solubility and a high intrinsic permeability. In humans, apixaban has a small volume of distribution (~0.1 kg), a half-life of ~12 hours, and a low peak-to-trough plasma concentration ratio (<4) following oral twice daily administration (Wong et al., 2011; Frost et al., 2013).

The intestine is a highly perfused organ supplied by the superior mesenteric artery that carries approximately 10–20% of cardiac output in mice, rats, rabbits, dogs, monkeys, and humans with 60% of mucosal blood flow supplying the epithelial cells of the villi (Davies and Morris, 1993; Matheson et al., 2000; Jamei et al., 2009). Drug Metabolism, Pfizer Worldwide Research and Development (T.C.G.).

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ABBREVIATIONS: AC, activated charcoal; apixaban, 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide; AUC, area under the plasma concentration versus time curve; BCRP (abcg2), breast cancer resistance protein; BDC, bile duct cannulation; CL, clearance; CLmax, maximal clearance; CLint, intestinal clearance; CLint/met, metabolism clearance; CLint/bil, biliary clearance; EER, enteroenteric recirculation; EHR, enterohepatic recirculation; F, bioavailability; GF-120918, N-[4-[2-(3,4-dihydro-6,7-dimethoxy-2(1H)-isoquinolinyl]ethyl]phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridinecarboxamide; GI, gastrointestinal tract; HP-β-CD, hydroxypropyl-β-cyclodextrin; HPLC, high-performance liquid chromatography; IC, intestinal clearance; IE, intestinal excretion; KO, knockout; LC/MS, liquid chromatography-mass spectrometry; MRT, mean residence time; P-gp, permeability-glycoprotein; RTR, renal tubule recirculation; Tmax, time to reach peak concentration; WT, wild type.
transporters and drug-metabolizing enzymes are heterogeneously expressed in the intestine, affecting drug absorption and bioavailability (Suzuki and Sugiyama, 2000; Kaminsky and Zhang, 2003; Paine et al., 2006). P-gp (P-glycoprotein; abcb1) and BCRP (breast cancer resistance protein; abcg2), two members of the ABC transporter superfamily, are highly expressed at the luminal brush border membranes of intestinal enterocytes, proximal tubular membranes in the kidney, and canalicular membranes of hepatocytes in the liver and play an important role in drug absorption, tissue distribution, and drug elimination (Maliepaard et al., 2001; Doyle and Ross, 2003; Unadkat et al., 2004; Mao and Unadkat, 2005; Krishnamurthy and Schuetz, 2006; Huls et al., 2008; Koshiba et al., 2008; Zhou, 2008; Vlaming et al., 2009; Giacomini et al., 2010). In human, the MDRI (P-gp) gene encodes a single drug transporting P-gp, whereas in mice and rats, the mdrla and mdrlb genes encode two P-glycoprotein isoforms, but mdrlb is not expressed in mouse intestine (Thibault et al., 1987; Schinkel et al., 1994). The expression of intestinal efflux transporters is region dependent, with the expression of P-gp generally reported to increase from proximal duodenum to distal ileum in rats and humans (Mouly and Paine, 2003; Englund et al., 2006; Berggren et al., 2007; Hilgendorf et al., 2007; MacLean et al., 2008; Haslam et al., 2011). Both BCRP expression and its efflux function reached to peak in the ileum in rat and mouse intestines (Enokizono et al., 2007; Haslam et al., 2011). Prior to dosing each animal (rat or dog) was weighed, randomized, and assigned a permanent identification number. During the acclimation period (at least 2 days prior to study initiation), the animals were housed in individual, suspended, stainless steel wire mesh cages. The animals were fasted overnight (at least 12 hour) prior to and through 4 hours following dose administration. The animal room was controlled to maintain a temperature of 75°F and 70% relative humidity, with a 12-hour light/dark cycle. The body weight of each animal was determined on the day before dosing. The doses administered were calculated based on the body weight of each animal. The formulations were administered to fasted animals from each group by oral gavage using a syringe with a gavage needle to deliver a dose at a target dose level or by intravenous doses. The amount of dose administered to each animal was determined by the difference in weights of the loaded dose syringe and needle prior to dose administration and the emptied syringe and needle after dose administration. Apixaban (5 mg/kg, 1 mg/ml in 0.5% Tween 80 in Labrafli) was administered via oral gavage to 4 male beagle dogs followed by a 10 ml flush of water. AC (250 mg/kg, 208 mg/ml water suspension) was administered via oral gavage in three separate treatments in a parallel crossover design followed by a 10 ml flush of water at 0.25, 1, or 3 hours postdose. Male dogs (intact n = 3 or BDC n = 2) were dosed via i.v. infusion (5 minutes) of [14C]apixaban (1 mg/kg, 0.5 ml/kg). AC suspensions were administered to BDC dogs at 250 mg/kg via oral gavage at 10, 90, 180, and 420 minutes after the [14C]apixaban dosing. Apixaban (4 mg/kg) was administered to WT, GF-120918-pretreated WT, P-gp-KO, and BCRP-KO rats via oral gavage. [14C]Apixaban (2 mg/kg) was administered to BDC WT, GF-120918-pretreated WT, P-gp-KO, and BCRP-KO rats via i.v. bolus administration. GF-120918 was administered orally (10 mg/kg, 2 ml/kg, 5 mg/ml) at 1 hour before apixaban dose in intact rats or at 1 hour before and...
8 hours after $[^{14}C]$apixaban dosing in BDC rats. AC suspensions were also administered to BDC rats at 250 mg/kg via implanted duodenal catheters at 30 minutes before and 5, 90, and 180 minutes after the $[^{14}C]$apixaban dosing.

Blood was collected at various time points (K$_2$EDTA as an anticoagulant) via venipuncture of a peripheral blood vessel in dogs and femoral veins catheters from rats. These samples were placed on ice immediately after collection and centrifuged within 30 minutes of collection to harvest plasma. Urine, bile, and feces were collected as described in Table 2.

Radioactivity Determination. Triplicate aliquots of the diluted dose solutions, plasma, urine, bile, and cage wash were analyzed for radioactivity concentrations. Radioactivity determinations were made with a Tri-Carb Model 3100 TR liquid scintillation counter (PerkinElmer) using 5 ml of scintillation fluid (Ultima Gold) per sample or 15 ml of Ecolite.

Fecal samples were homogenized in four volumes of water. Triplicate aliquots (0.40–1.1 g) of fecal and gastrointestinal tract (GI) tract homogenates were placed into preweighed Combusto-cones and pads and allowed to air-dry overnight. The samples were then combusted and analyzed for radioactivity. A model 307 Tri-Carb Sample Oxidizer, equipped with an Oximate-80 Robotic Automatic Sampler (PerkinElmer), was used for combustion of fecal homogenates. The $^{14}$CO$_2$ generated from dosing of $[^{14}C]$apixaban was trapped in Carbo-Sorb E (7 ml) and mixed with PermaFluor E+ (8 ml) as the scintillant to determine radioactivity concentrations using a Tri-Carb Model 3100 TR liquid scintillation counter (PerkinElmer). The oxidation efficiency of the oxidizer was greater than 97%.

Metabolite Profiling. Metabolites in plasma, bile, and urine and fecal samples were profiled as described previously (Raghavan et al., 2009; Wang et al., 2011; Zhang et al., 2009a). Pooled plasma, urine, bile, or fecal homogenate samples were prepared by aliquoting appropriate volumes or weights of samples from each time interval. The pooled urine and bile samples were directly analyzed to obtain metabolite profiles following centrifugation at 1500 g for 15 minutes at 4°C. The pooled fecal samples (about 1 ml) were mixed with 2 ml of methanol and shaken for 10 minutes. The mixtures were centrifuged at 1500 g for 10 minutes at 4°C. The supernatants were transferred to clean tubes. The pellets were extracted twice with 2 ml of methanol (2 x 2 ml). All supernatants were combined and evaporated to dryness under nitrogen, and the residues were reconstituted in 400 μl of 25% methanol in water. Metabolite profiling for pooled urine, bile, and feces, and dose analysis was performed on a Surveyor HPLC system equipped with a $\beta$-RAM radioactivity flow detector (Model 4; IN/US Systems, Inc., Tampa, FL). The radioactivity detector, $\beta$-RAM, was equipped with a 100 μl flow cell and was operated using scintillation cocktail (Ultima Flo M) delivered at a flow rate of 1.0 ml/min. Chromatography was accomplished on a Phenomenex Luna C18, 4.6 x 250 mm, 3 μm (Torrance, CA) for the apixaban studies. The mobile phase for apixaban sample profiling consisted of two solvents: A, 0.05% trifluoroacetic acid and B, 0.05% trifluoroacetic acid in acetonitrile; the gradient was as:

**TABLE 1**

<table>
<thead>
<tr>
<th>Species (Route)</th>
<th>Test Article</th>
<th>Vehicle</th>
<th>Dose Concentration</th>
<th>S.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog (oral)</td>
<td>Apixaban</td>
<td>0.5% Tween 80 in Labrafil</td>
<td>mg/ml</td>
<td>1</td>
</tr>
<tr>
<td>Dog (i.v.)</td>
<td>$[^{14}C]$apixaban</td>
<td>30% HP-$\beta$-CD in 10 mM phosphate buffer (pH 7.2)</td>
<td>mg/ml</td>
<td>2</td>
</tr>
<tr>
<td>Rat (oral)</td>
<td>Apixaban</td>
<td>30% HP-$\beta$-CD in 10 mM phosphate buffer (pH 7.2)</td>
<td>mg/ml</td>
<td>4</td>
</tr>
<tr>
<td>Rat (i.v.)</td>
<td>$[^{14}C]$apixaban</td>
<td>30% HP-$\beta$-CD in 10 mM phosphate buffer (pH 7.2)</td>
<td>mg/ml</td>
<td>2</td>
</tr>
<tr>
<td>Rat (oral)</td>
<td>GF-120918</td>
<td>30% HP-$\beta$-CD in 10 mM phosphate buffer (pH 7.2)</td>
<td>mg/ml</td>
<td>5</td>
</tr>
<tr>
<td>Rat (oral)</td>
<td>GF-120918</td>
<td>N-methyl-2-pyridoline/saline (1:1)</td>
<td>mg/ml</td>
<td>5</td>
</tr>
<tr>
<td>Rat, dog</td>
<td>AC</td>
<td>Purified water</td>
<td>mg/ml</td>
<td>250/208</td>
</tr>
</tbody>
</table>

AC, activated charcoal; HP-$\beta$-CD, hydroxypropyl-$\beta$-cyclodextrin; NA, not applicable; S.A, specific activity

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**Fig. 2.** EER, EHR, and RTR (A) and the impact of activated charcoal (B). Arrows indicate drug flow and narrow arrows indicate less and gray arrows indicate reduced drug flow by activated charcoal. PO, orally.
The internal standard. The range of quantitation of apixaban in plasma under system (Shimadzu Scientific Instruments, Kyoto, Japan) was performed empore C8-SD 96-well solid extraction plate, HPLC on a Shimadzu LC-10AT total radioactivity in plasma, urine, bile, and feces. In rats, unchanged \[{\text{14C}}\]apixaban accounted for 96.4, 93.7, 91.2% in urine; 20.7, 34.8, and 18% in plasma, urine, bile, feces, (0–48 h) respectively.

Quantitation of Apixaban Concentrations. The concentrations of apixaban in plasma were determined by a validated liquid chromatography-tandem mass spectrometry method using 0.05 ml of plasma and \[{\text{13C}}\]apixaban as the internal standard. The range of quantitation of apixaban in plasma under these conditions was 1–1000 ng/ml. After solid phase extraction on a 3M ACE 3 C18 column (4.6 mm × 150 mm). The mobile phase consisted of two solvents: A, 0.4% formic acid in water, pH 3.2, and B, 100% acetonitrile. The mass spectrometer (Thermo Scientific, San Jose, CA). Samples were analyzed in the positive ionization mode, and the capillary temperature was set at 300°C.

An alternative HPLC system was a Hewlett Packard Agilent 1100 series system equipped with two pumps, an autoinjector, a ultraviolet detector, and an ACE 3 C18 column (4.6 mm × 150 mm). The mobile phase consisted of two solvents: A, 0.4% formic acid in water, pH 3.2, and B, 100% acetonitrile. The gradient was as follows: solvent B started at 5%, then linearly increased to 20% at 5 minutes, to 30% at 50 minutes, to 90% at 65 minutes, and to 5% at 36 minutes with a HPLC effluent of 0.7 ml/min. The peak plasma concentration (\(C_{\text{max}}\)) and the time to reach peak concentration (\(T_{\text{max}}\)) were recorded directly from experimental observations. The area under the plasma concentration versus time curve from 0 to T (AUC\(_{0–T}\)), where T equals the time of the last measured plasma concentration, was calculated by a combination of conventional trapezoidal and log-trapezoidal methods by Kinetic v4.4 (InnaPhase Corp., Philadelphia, PA) or by using WinNonlin Professional Edition, version 4.1 (Pharsight, Mountain View, CA). AUC to infinity (AUC\(_{0–\infty}\)) was determined by the sum of AUC\(_{0–T}\) and the extrapolated area. The extrapolated area was determined by dividing the last measured concentration by the slope of the terminal log-linear phase, which was <8% for all animal groups used in this study upon sample collection of sufficient data points. The terminal slope \((\beta)\) of the plasma concentration-time profile was determined by the method of least squares (log-linear regression of at least three data points). The terminal half-life was estimated as ln2/β.

For metabolite identification, the HPLC flow was directed to LTQ ion trap mass spectrometer (Thermo Scientific, San Jose, CA). Samples were analyzed in the positive ionization mode, and the capillary temperature was set at 300°C.

The flow rate of nitrogen gas, spray current, and voltages were adjusted to give maximum sensitivity for apixaban.

Enteroenteric and Renal Tubule Recirculation of Apixaban 909

Enteroenteric and Renal Tubule Recirculation of Apixaban 909

Table 2 lists the radioactive dose recovery from BDC dogs following a 5 mg/kg oral dose with and without subsequent single administration of AC were shown in Fig. 3, A and B, and single-dose pharmacokinetic parameters are shown in Table 3. The apixaban pharmacokinetic data were compared in the same dog for the control pharmacokinetic data were compared in the same dog for the control and AC treatment separated by 2 weeks. When AC was administered 0.25 (15 minutes), 1, and 3 hours after apixaban, apixaban AUC\(_{0–24}\) was 24, 19, and 37%, respectively, lower compared with apixaban alone. Apixaban \(C_{\text{max}}\) were not markedly affected by administration of AC at any of the time points. Apixaban \(T_{\text{max}}\) was 5.6 hours when administered alone, but decreased to <4 hours when AC was administered 1 and 3 hours after apixaban, respectively, with a corresponding increase of the systemic clearance (29–66%) and decrease in the mean residence time (MRT) and the \(C_{\text{ss}}\) values (\(C_{\text{min}}\)).

BDC Dog Studies. Table 4 lists the radioactive dose recovery from BDC dogs following i.v. administration of \[{\text{13C}}\]apixaban (1 mg/kg) with and without administration of AC. Approximately 40–50% of the dose was recovered in feces of BDC dogs, indicating the drug

Data Analysis. The plasma concentration versus time data for apixaban were analyzed with a noncompartmental method (Perrier and Gibaldi, 1982). The peak plasma concentration (\(C_{\text{max}}\)) and the time to reach peak concentration (\(T_{\text{max}}\)) were recorded directly from experimental observations. The area under the plasma concentration versus time curve from 0 to T (AUC\(_{0–T}\)), where T equals the time of the last measured plasma concentration, was calculated by a combination of conventional trapezoidal and log-trapezoidal methods by Kinetic v4.4 (InnaPhase Corp., Philadelphia, PA) or by using WinNonlin Professional Edition, version 4.1 (Pharsight, Mountain View, CA). AUC to infinity (AUC\(_{0–\infty}\)) was determined by the sum of AUC\(_{0–T}\) and the extrapolated area. The extrapolated area was determined by dividing the last measured concentration by the slope of the terminal log-linear phase, which was <8% for all animal groups used in this study upon sample collection of sufficient data points. The terminal slope \((\beta)\) of the plasma concentration-time profile was determined by the method of least squares (log-linear regression of at least three data points). The terminal half-life was estimated as ln2/β. The clearance via intestinal excretion was estimated as: \(\text{CLR}_{\text{int}} = \text{amount of apixaban in feces of BDC animals/AUC. Urinary clearance (CLR)}\) was calculated in a similar manner based on data obtained from the urine collections. The total clearance via biliary excretion and metabolism (\(\text{CLR}_{\text{biliary+metabolism}}\)) was estimated by using: \(\text{CLR}_{\text{biliary+metabolism}} = \text{CLR}_{\text{total}} – \text{CLR}_{\text{int}} – \text{CLR}_{\text{R}}\).

\[\begin{array}{|c|c|c|c|c|c|}
\hline
\text{Test Article} & \text{Species (n), Type} & \text{Dosing Routes} & \text{Other Treatments} & \text{Dose (mg/kg)} & \text{Sample Collection} \\
\hline
\text{Apixaban} & \text{Dog (4), intact} & \text{Oral} & \text{None, charcoal at 0.25, 1, and 3 h} & 5 & \text{Plasma (0.5, 1, 2, 4, 6, 8, 12, 14, 20, 24 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Dog (3), Intact} & \text{i.v.} & \text{None} & 1 & \text{Urine, bile, feces, (0–72 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Dog (2), BDC} & \text{i.v.} & \text{None, charcoal} & 1 & \text{Plasma, urine, bile, feces, (0–72 h)} \\
\hline
\text{Apixaban} & \text{Rat (3), WT, intact} & \text{Oral} & \text{None} & 4 & \text{Plasma (0.5, 1, 2, 3, 4, 5, 7, 10, 24 h)} \\
\hline
\text{Apixaban} & \text{Rat (3), P.gp-KO, intact} & \text{Oral} & \text{None} & 4 & \text{Plasma (0.5, 1, 2, 3, 4, 5, 7, 10, 24 h)} \\
\hline
\text{Apixaban} & \text{Rat (3), BCRP-KO, intact} & \text{Oral} & \text{None} & 4 & \text{Plasma (0.5, 1, 2, 3, 4, 5, 7, 10, 24 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Rat (3), WT, intact} & \text{i.v.} & \text{None} & 2 & \text{Urine, bile, feces, (0–24 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Rat (3), WT, BDC} & \text{i.v.} & \text{None} & 2 & \text{Plasma, urine, bile, feces, (0–48 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Rat (3), WT, BDC} & \text{i.v.} & \text{Charcoal} & 2 & \text{Plasma, urine, bile, feces, (0–48 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Rat (3), P.gp-KO, BDC} & \text{i.v.} & \text{None} & 2 & \text{Plasma, urine, bile, feces, (0–48 h)} \\
\hline
\text{\^[13C]apixaban} & \text{Rat (3), BCRP-KO, BDC} & \text{i.v.} & \text{None} & 2 & \text{Plasma, urine, bile, feces, (0–48 h)} \\
\hline
\end{array}\]

AC, activated charcoal; BCRP, breast cancer resistance protein; BDC, bile duct cannulation; KO, knockout; P-gp, P-glycoprotein; WT, wild-type.

1 Time points: 0.083, 0.25, 0.5, 1, 2, 3, 5, 8, 24 hours.
2 Time points: 0.083, 0.25, 1, 2, 4, 6, 8, 12, 24 hours.
3 Charcoal (via gavage) dosing time: 10, 90, 180, 420 minutes.
4 Charcoal (via intraduodenal route) dosing time: –30, 5, 90, 180 minutes.
5 i.v.: infusion time: 5 minutes.
was directly excreted into the gut and eliminated in the feces. Upon treatment with AC, the fecal recovery was increased in BDC dogs with no marked effect on the urinary elimination, and apixaban AUC was reduced by approximately 19–39% and apparent systemic clearance of apixaban was increased by 20–60% (Fig. 4A; Table 3). The increased fecal elimination with AC in BDC dogs corresponded to a >50% increase in intestinal clearance (IC), suggesting that AC prevents reabsorption of apixaban and resulted in the increases of IE through interruption of enteroenteric recirculation. In dogs, the peak-to-trough concentration ratio (Cmax/C24) after oral administration increased from 15 to >30 with AC treatments. The half-life (T1/2) and MRT were lower in dogs after i.v. versus oral administration. Approximately 10–20% of dose was recovered in the 0-72 hours urine collection from BDC dogs. For comparison, urinary recovery was also similar in intact dogs following i.v. (Table 4) and oral administration of [14C]apixaban (Zhang et al., 2009a).

**BDC Rat Studies.** IE of apixaban was further investigated in BDC rats following i.v. administration of [14C]apixaban (2 mg/kg). Figure 4B describes the apixaban plasma concentration-time profiles in BDC rats with and without administration of AC or in the presence of GF-120918, a BCRP and P-gp inhibitor (Jonker et al., 2000; Matsson et al., 2009). The radioactive dose recovery values in bile, urine, and feces and pharmacokinetic parameters of apixaban in the BDC rats are listed in Tables 5 and 6, respectively. Approximately 21% of dose was found in the feces of BDC rats following i.v. administration of [14C]apixaban, suggesting direct IE of apixaban in rats, which led to IC representing 21% of the total systemic clearance. Administration of GF-120918 reduced the fecal dose recovery of apixaban by 34%, and the administration of AC increased the fecal recovery by approximately 36%. The AUC0–<i>∞</i> of apixaban was increased (100%) with GF-120918 and decreased (28%) with AC (Table 6). The IC and systemic clearance were decreased 66 and 50% by GF-120918 treatment and increased 80 and 42% by AC treatment, respectively. These data clearly demonstrated that inhibition of intestinal efflux transporters decreases the systemic clearance and IC of apixaban leading to the reduced IC, whereas AC increased both

![Fig. 3. Apixaban plasma concentration-time profiles in dogs (<i>n</i> = 4) following a 5 mg/kg oral dose of apixaban with and without subsequent single administration of activated charcoal. (A) Log scale; (B) linear scale.](image-url)

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Pharmacokinetic parameters of male intact and bile duct-cannulated beagle dogs following a single oral dose of apixaban or i.v. dose of [14C]apixaban with and without oral administration of activated charcoal</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Dosing Route</th>
<th>Oral Dose in Intact Dogs (&lt;i&gt;n&lt;/i&gt; = 4)</th>
<th>IV. Dose in BDC Dogs (&lt;i&gt;n&lt;/i&gt; = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (5 mg/kg)</td>
<td>AC (in the same dog) (1 mg/kg)</td>
</tr>
<tr>
<td>AUC0–&lt;i&gt;∞&lt;/i&gt;</td>
<td>78.6 ± 7.0 (NA)</td>
<td>24.8, 9.30 (NA)</td>
</tr>
<tr>
<td></td>
<td>59.5 ± 4.6 (24%)</td>
<td>20.1, 5.60 (19, 39%)</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>9.35 ± 1.33</td>
<td>6.97, 4.43</td>
</tr>
<tr>
<td>C&lt;sub&gt;x&lt;/sub&gt;</td>
<td>5.23 ± 1.02</td>
<td>0.98, 0.35</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>5.7 ± 2.2</td>
<td>3.5, 2.9</td>
</tr>
<tr>
<td>CLF, CL (ml/h/kg)</td>
<td>60 ± 16</td>
<td>4.0, 1.1</td>
</tr>
<tr>
<td>Vss/F, Vs (l/kg)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>8.5 ± 2.3</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>F&lt;sup)&gt;a&lt;/sup&gt;</td>
<td>93%</td>
<td>0.96, 0.44</td>
</tr>
<tr>
<td>Cmax/C24 (&lt;i&gt;C&lt;/i&gt;&lt;sub&gt;x&lt;/sub&gt; i.v.)</td>
<td>15</td>
<td>17.3, 12.4 (43-47%)</td>
</tr>
<tr>
<td>CL&lt;sub&gt;ur&lt;/sub&gt; (ml/h/kg),% total&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>17.2, 52.2 (43-47%)</td>
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<tr>
<td>CL&lt;sub&gt;ir&lt;/sub&gt; (ml/h/kg),% total&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>31.2, 133 (62-74%)</td>
</tr>
<tr>
<td>CL&lt;sub&gt;total&lt;/sub&gt; (ml/h/kg),% total&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>17.3, 37.3 (43-47%)</td>
</tr>
</tbody>
</table>

AC, activated charcoal; AUC, area under the plasma concentration versus time curve; CL, clearance; F, bioavailability; MRT, mean residence time; NA, not available; ND, not determined.

<sup>a</sup> Cmax was last detectable time point for i.v. dosing or last determined time point for oral dosing.

<sup>b</sup> Both CL<sub>x</sub> and C24 were listed for oral administration.

<sup>c</sup> Bioavailability might be overestimated because the AUC values of bile-duct cannulated animals were used.

<sup>d</sup> Clearance was estimated from urinary dose recovery from 0-72 hour collections.

<sup>e</sup> The protein binding of apixaban in dog plasma was 92%, therefore, glomerular filtration rate <i>x</i>Fu = 368 x 0.08 = 29.4 ml/h/kg.
The increase in the P-gp-KO rats was less (from 21 to 25%) (Table 6). The urinary excretion was decreased from 50 to 35% in the BCRP-KO rats but by 300% in the BCRP-KO rats compared with the wild-type rats. As shown in Fig. 5 and Table 6, apixaban exposure (AUC) increased by 45 and 66%, respectively, in both P-gp-KO and BCRP-KO rats compared with the wild-type rats (Table 6). The contributions of P-gp and BCRP in governing apixaban disposition were also examined after oral doses to P-gp-KO and BCRP-KO rats with a transporter inhibitor. As shown in Fig. 5 and Table 6, apixaban exposure (AUC) increased by 100% in the P-gp-KO rats compared with the wild-type rats (Table 6). The apixaban AUC0–24h increased by 45 and 66%, respectively, in both P-gp-KO and BCRP-KO rats compared with the wild-type rats (Table 6). The contributions of P-gp and BCRP in governing apixaban disposition were also examined after oral doses to P-gp-KO and BCRP-KO rats or with a transporter inhibitor. As shown in Fig. 5 and Table 6, apixaban exposure (AUC) increased by 100% in P-gp-KO rats but by 300% in the BCRP-KO rats compared with the wild-type rats after oral administration, predominately through an increase in Cmax. GF-120918 increased apixaban expositions (AUC) by nearly 400%. Compared with the plasma concentration-time profiles of apixaban in dogs (Fig. 3) and BCRP-KO rats (Fig. 5) after oral administration, apixaban showed a broad peak and even multiple peaks after oral administration in rats. The broad peak complexity of apixaban was more obvious in P-gp-KO rats than BCRP-KO rats. The apparent flip-flop pharmacokinetic profile of apixaban in rats (Table 5) as well as in dogs (Table 3) was characterized by a short T1/2 value after i.v. than oral administration and the increase of Cmax/Cmin ratios after oral versus i.v. administration, which was reduced in the P-gp-KO and BCRP-KO rats compared with the wild-type rats (Fig. 5; Table 5). The urinary excretion was approximately 40–45% of dose in both intact and BDC rats following i.v. administration of [14C]apixaban, indicating that bile duct cannulation did not affect the urinary excretion of apixaban. However, the urinary excretion after i.v. administration (40–45% of dose) was much higher compared with approximately 10% of dose after oral administration (Zhang et al., 2009a). Following oral administration in rats, P-gp-KO and BCRP-KO as well as GF-120918 pretreatments generally increased absorption, decreased volume of distribution and MRT, and decreased the apparent systemic clearance values of apixaban (Table 5).

**Discussion**

Approximately 20–50% of the i.v.-administered [14C]apixaban dose recovered in the feces of BDC rats and dogs confirmed that there was direct excretion of the drug from blood into the GI. The findings prompted further investigation of the mechanism of IE and the interplay of IE with EER and enterohepatic recirculation (her) (Fig. 2). A key to the finding of IE was an examination of efflux transporter involvement in the intestinal disposition of apixaban, which led to additional understanding of the involvement of RTR in apixaban disposition.

![Fig. 4](https://example.com/apixaban_plasma_profiles.png)

**Fig. 4.** Apixaban plasma concentration-time profiles in BDC dogs (A; n = 2) following a 1 mg/kg i.v. dose and in BDC rats (B; n = 3) following a 1 mg/kg i.v. dose of [14C]apixaban with and without oral administration of activated charcoal and GF-120918.

---

**TABLE 4**

Percent recovery of radioactivity (± S.D.) following a single 1 mg/kg i.v. dose of [14C]apixaban to male intact and bile duct-cannulated beagle dogs

<table>
<thead>
<tr>
<th>Compound and Dose Route</th>
<th>Dog Type</th>
<th>Dose Recovery (% of Dose in 0–72 h Collections)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>[14C]apixaban (i.v.)</td>
<td>BDC, n = 2</td>
<td>13.5, 19.1</td>
</tr>
<tr>
<td>[14C]apixaban (i.v.) and charcoal</td>
<td>BDC, n = 2</td>
<td>16.3, 8.1</td>
</tr>
<tr>
<td>[14C]apixaban (i.v.)</td>
<td>Intact, n = 3</td>
<td>9.9 ± 7.9</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>Intact, n = 3</td>
<td>8.8</td>
</tr>
</tbody>
</table>

BDC, bile duct cannulation; NA, not available.

* A crossover study with dosing separated by 1 week.

* Oral dose was 5 mg/kg and collections were 0–168 hours for 3 intact dogs (Zhang et al., 2009a) and listed here for comparison.
Direct IE led to significant fecal elimination in rats and dogs. Following i.v. administration of [14C]apixaban to BDC rats and dogs, approximately 20–50% of dose was recovered in feces, resulting in IC that represented 20–50% of the total systemic clearance. The reduction of i.v.-dosed apixaban AUC by AC in BDC rats and dogs with the increased fecal recovery and IC of apixaban clearly demonstrated that AC prevented reabsorption of apixaban that was excreted into intestinal lumen and so lead to interruption of EER. Orally administered GF-120918, a dual P-gp and BCRP inhibitor, reduced IE of apixaban in rats without affecting other elimination pathways, suggesting that these two transporters facilitated apixaban IE. AC administration was effective at 3 hours in dogs after an apixaban oral dose, when the majority of the apixaban absorption has passed, which was consistent with an interruption of apixaban reabsorption proceeds by IE. Similar to the charcoal effects in dogs, apixaban $T_{\text{max}}$ and $C_{\text{max}}$ were not affected by AC administration in humans. A decrease in apixaban AUC of approximately 50 and 27% and a decrease of $T_{1/2}$ from 13.4 hours to 5.3 and 4.9 hours was observed when AC was administered at 2 and 6 hours postdose. The late charcoal effect is consistent with IE in humans. The 34% of dose recovered as apixaban in feces of humans following oral administration of [14C]apixaban (the human absorption, distribution, metabolism, and excretion study) is also consistent with the apixaban IE (Raghavan et al., 2009). The quantitative difference in IE between species is difficult to predict as it would be impacted by the physiological and biochemical differences in expression of transporters and drug metabolizing enzymes in the different species, although the GI tracts of rats and humans seem to be similar in the absorption properties of many drugs (Zhao et al., 2003; Cao et al., 2006) and intestinal BCRP expression profiles are similar between dog and human (Haller et al., 2012). The extent of IE will also depend on other elimination pathways such as metabolism, glomerular filtration, and biliary excretion. Metabolism appeared to be more important for apixaban elimination in humans (25% of dose) than in rats and dogs (<15%) (Zhang et al., 2009a). Urinary excretion of apixaban in rats (40%) was higher than that in dogs and humans. IE apparently accounts for approximately >20, >42, and >27% of clearance in rats, dogs, and humans, respectively, based on the BDC rat and dog study and the human mass balance (Raghavan et al., 2009) as well as the AC study in humans. IE as an important elimination pathway of apixaban, in addition to metabolism (25%) and urinary excretion (27%) in humans, would reduce the impacts of intrinsic (renal or hepatic impairment with <40% increase of exposures in severely impaired subjects) and extrinsic (drug-drug interactions with less than twofold changes in exposure.

### TABLE 5
Percent recoveries of radioactive dose (± S.D.) following a single i.v. dose of [14C]apixaban to male bile duct-cannulated Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Compound and Dose Route</th>
<th>Rat Type</th>
<th>Dose Recovery (% of Dose in 0–24 h Collections)</th>
<th>Urine</th>
<th>Bile</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]apixaban (i.v.)</td>
<td>WT, BDC, n = 3</td>
<td>49.5 ± 4.2</td>
<td>10.7 ± 1.1</td>
<td>21.1 ± 5.2</td>
<td>82.3 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (i.v.) and GF-120918</td>
<td>WT, BDC, n = 3</td>
<td>50.6 ± 3.1</td>
<td>14.7 ± 2.2</td>
<td>13.8 ± 2.4</td>
<td>79.1 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (i.v.) and Charcoal</td>
<td>WT, BDC, n = 3</td>
<td>40.7 ± 2.6</td>
<td>15.7 ± 1.9</td>
<td>28.8 ± 2.5</td>
<td>85.2 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (i.v.) P-gp-KO, BDC, n = 3</td>
<td>44.3 ± 4.6</td>
<td>14.1 ± 2.7</td>
<td>24.9 ± 9.1</td>
<td>83.2 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (i.v.) BCRP-KO, BDC, n = 3</td>
<td>35.5 ± 0.4</td>
<td>12.7 ± 3.4</td>
<td>34.1 ± 4.3</td>
<td>82.3 ± 5.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (oral)*</td>
<td>WT, intact, n = 3</td>
<td>51.3 ± 5.1</td>
<td>NA</td>
<td>41.1 ± 1.9</td>
<td>92.4 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>[14C]apixaban (oral)*</td>
<td>WT, BDC, n = 3</td>
<td>13.4</td>
<td>NA</td>
<td>74.0</td>
<td>87.4</td>
<td></td>
</tr>
</tbody>
</table>

BCRP, breast cancer resistance protein; BDC, bile duct cannulation; KO, knockout; NA, not available; P-gp, P-glycoprotein; WT, wild-type.

* Oral dose was 30 mg/kg and collections were 0–168 h for 3 intact and 0–48 h for 3 BDC rats (Zhang et al., 2009a) and listed here for comparison.

### TABLE 6
Pharmacokinetic parameters of apixaban following a single oral dose of apixaban or i.v. dose of [14C]apixaban to male bile duct-cannulated Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Oral Dose in Intact Rats (4 mg/kg) (n = 3)</th>
<th>Intraoral Dose in BDC Rats (2 mg/kg) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound and Dose Route</strong></td>
<td><strong>Urine</strong></td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>WT Control</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>P-gp-KO</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>BCRP-KO</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>WT GF-120918</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>WT Control</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>P-gp-KO</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>BCRP-KO</td>
</tr>
<tr>
<td>[14C]apixaban (oral)</td>
<td>WT GF-120918</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration versus time curve; BCRP, breast cancer resistance protein; BDC, bile duct cannulation; CL, clearance; F, bioavailability; KO, knockout; MRT, mean residence time; NA, not available; ND, not determined; P-gp, P-glycoprotein; WT, wild-type.

* Samples were taken at different time points.

* Bioavailability was underestimated because the AUC values of bile duct-cannulated animals were used where enterohepatic recirculation could not be considered. The >100% bioavailability in the BCRP-KO rats and after the treatments with GF-120918 reflected the increased absorption in transporter knockout rats and the increased urinary clearance of apixaban after i.v. administration of BDC rats compared with oral administration of intact rats in addition to elimination of potential low level of enterohepatic recirculation in intact rats, both of which reduced the apparent apixaban AUC after i.v. administration.

* The protein binding of apixaban in rat plasma was 96%, therefore, glomerular filtration rate $\times F_u = 0.31 \times 0.04 = 0.012$ lb/kg.

A compound with high solubility and high intrinsic permeability such as apixaban can be absorbed rapidly after oral administration at the proximal intestine, partially escaping the barrier function of P-gp and BCRP that are highly expressed in the small intestines (Mori et al., 2008). Apixaban appears to fit that profile as it does have a relatively short $T_{\text{max}}$. AC administration at various times (0.25–6 hours) post-apixaban oral dose in dogs (solution formulation) and humans (tablet formulation) did not significantly affect the $C_{\text{max}}$ or $T_{\text{max}}$ of apixaban as apixaban was rapidly absorbed. The lack of an effect on $C_{\text{max}}$ led to the conclusion that the impact of AC is not on the initial absorption phase but on the IE/EER phase (Fig. 2). Timely administration of AC could sequester the drug that has been excreted into the lumen and prevention of reabsorption. Administration of AC 0.25 hour after drug dose in dogs was less effective at reducing the apixaban AUC versus 3 hours because AC did not have sufficient opportunity to mix with apixaban preabsorption and transited the GI tract prior to extensive IE; therefore, there was actually decreased effective contact with the drug when charcoal was dosed at 0.25 hour. Considering the small intestinal transit time of 2–4 hours for substances such as charcoal in dogs (Orisakwe and Ogbonna, 1993; Parrott and Lave, 2008; Abuasal et al., 2012), administration of AC at 2–4 hours after the drug dose, when apixaban initial absorption in the upper GI tract is complete and the IE phase is beginning, may be the most effective timing to remove the drug from the intestine. In the presence of AC, the shorter half-life and greater clearance in dogs and humans after oral dose of apixaban and in rats and dogs after i.v. administration of apixaban supports that AC increases the apparent systemic clearance of apixaban by 20–40% and IC by 20–60%. Therefore, AC removes apixaban from the body by preventing its reabsorption. AC has been shown to block EHR of some drugs in human and animal studies (Gadgil et al., 1982; Heimer and Englund, 1986; Roberts et al., 2002; Stass et al., 2005). Additionally, the shorter half-life and lower exposures of moxifloxacin and phenobarbital upon administration of AC in humans and rabbits following i.v. drug administration has been ascribed to AC blocking enteroenteric recycling of the drugs (Wakabayashi et al., 1994; Stass et al., 2005).

EER is a complex and dynamic process of concentration gradient-dependent transcellular passive diffusion, transporters-mediated transport, and segment-dependent reabsorption, operating in the same or opposite direction to intestinal mobility and intestinal transit. Apixaban molecules that undergo IE can be reabsorbed, although reabsorption might occur at a more distal segment of the intestine where there is a lower level of transporter expression. The drug secreted into the lumen serves as a secondary source of drug for absorption. Therefore, the intestine is also an organ for drug distribution and redistribution as well as absorption and clearance. Apixaban EER, along with EHR and RTR, are represented in Fig. 2. Due to the reabsorption, IE may or may not lead to a measurable level of fecal elimination. The interplay between drug metabolizing enzymes and transporters involved in EER as well as EHR and RTR could serve to enhance metabolism (Wu and Benet, 2005; Zhang et al., 2009b) because the drug eliminated into intestines is subject to further metabolism in the intestine and liver upon reabsorption. Despite extensive IE of apixaban, >50% of dose was recovered as parent drug from the human and animal species because of its metabolic stability. EER is a continuous excretion and reabsorption process and may or may not lead to a broad peak around $T_{\text{max}}$ in the plasma concentration time profile through multiple phases of distribution and elimination. This is different from EHR, which depends on the gallbladder-emptying time and often leads to a secondary drug peak in the plasma concentration versus time profile at 4–8 hour post-drug dose (Roberts et al., 2002).

Similar to intestinal excretion and reabsorption (EER), apixaban undergoes RTR (Fig. 2). Renal excretion is the result of glomerular filtration of unbound apixaban, tubular secretion, and tubular reabsorption (Fig. 2). Urinary elimination accounted for approximately 50% of the apixaban dose, and renal clearance represented approximately 50% of the systemic clearance in rats. On the basis of glomerular filtration rate and apixaban plasma protein binding (He et al., 2011), apixaban clearly shows active urinary excretion in rats. Conversely, urinary elimination only accounted for approximately 10% of the apixaban dose in dogs, and the renal clearance (~20 ml/h/kg) represents 13–19% of the systemic clearance, which would argue for a low level of net tubular reabsorption (glomerular filtration rate × $F_{\text{u}}$ = 29 ml/h/kg). Urinary elimination in humans was moderate (27% of dose) (Raghavan et al., 2009), and the renal clearance of apixaban is similar to the glomerular filtration rate likely due to the cancellation of active secretion versus reabsorption.

The renal elimination of apixaban was significantly reduced in the P-gp-KO and especially BCRP-KO rats following an i.v. dose, demonstrating that active transport is involved in apixaban renal elimination.

Fig. 5. Apixaban plasma concentration-time profiles in wild-type, P-gp-KO, and BCRP-KO rats ($n = 3$) following an intravenous dose at (2 mg/kg) and oral administration at 4 mg/kg of apixaban. (A) Linear scale; (B) log scale.
in rats. Knockout of a transporter would decrease active transport export, leading to the reduced urinary elimination. The reduced renal elimination led to the increased compensatory intestinal elimination of apixaban because knockout of P-gp or BCRP still leaves an alternative transporter in the intestine for apixaban efflux in addition to passive diffusion through loosely packed intestinal epithelium layer (Meier et al., 2006). Due to interference of renal excretion changes in the P-gp-KO or BCRP-KO rats, it was not possible to evaluate the contribution of P-gp or BCRP to apixaban IE directly; however, when apixaban was dosed orally, BCRP-KO rats showed an approximately twofold higher effect to increase the apixaban exposure versus the P-gp-KO rats. Therefore, BCRP plays a more important role than P-gp in apixaban absorption. IE of apixaban was markedly reduced by application of the P-gp and BCRP inhibitor GF-120918 in rats. The low level of BCRP expression in the human tubular epithelium cells (Huls et al., 2008) would suggest less impact of the transporter on apixaban urinary elimination in humans than rats.

In contrast to EER and RTR, EHR would not be as high for apixaban because biliary excretion is a minor elimination pathway for apixaban in rat and dog and likely in human. The biliary elimination of apixaban was not markedly affected by the transporter knockout or the transporter inhibitor mainly due to the fact that liver uptake is the driving force for the biliary elimination (Watanabe et al., 2009; Fenner et al., 2012; Varma et al., 2012) and apixaban was not a substrate for hepatic uptake transporters OATP1B1/3 or OAT1/3 (unpublished data).

The efflux of apixaban by P-gp and BCRP in the upper GI tract would delay apparent absorption of a fraction of apixaban dose, which could be a mechanism limiting overall rate of absorption, so $C_{\text{max}}$ is low after oral administration of apixaban. The increased $C_{\text{max}}$ values in the P-gp-KO and BCRP-KO rats have demonstrated the importance of intestinal efflux transporters in limiting apixaban absorption. The fraction for the reabsorption of apixaban in the duodenum, jejunum, and upper ileum would also be limited by the high levels of P-gp and BCRP expression, which could contribute to the broad peaks of apixaban. Both the recycling mechanisms, IE-intestinal reabsorption (EER) and glomerular filtration-tubular reabsorption (RTR), prolong apparent elimination half-life through decreasing apparent clearance of apixaban. The recycling mechanisms together with the metabolic stability of apixaban slowed down the decline of $C_{\text{min}}$. Therefore, the low peak-to-trough plasma concentration ratio of apixaban is consistent with intestinal efflux-limited absorption that controls the $C_{\text{max}}$ and the recycling mechanisms coupled with metabolic stability that controls the $C_{\text{min}}$. Furthermore, the recycling mechanisms (EER and RTR) of apixaban contributed to a half-life of 12 hours for apixaban despite its small volume of distribution and being subject to glomerular filtration. Different from the absorption-limiting flip-flop kinetics examples (Yanez et al., 2011), the apparent flip-flop kinetics of apixaban in rats is the result of the combined effects of intestinal BCRP/P-gp-limited absorption and RTR/EER recycling.

Taken together, AC application in BDC animals was a useful method to study IE and EER, which demonstrates that interruption of EER supports reduction of apixaban exposure by AC. IE has not been widely applied to understand dispositional profiles of drugs although studies have been reported to assess IE of drugs that are substrates of P-gp or BCRP using in vitro permeability assays, ex vivo segmental intestine excretion, and activated charcoal techniques (Mayer et al., 1996; Sparreboom et al., 1997; Gramatte and Oertel, 1999). With increasing use of physiologically based pharmacokinetic modeling to predict clinical profiles and drug-drug interaction potential that require accurate inputs of drug-related as well as physiological parameters (Bolger et al., 2009; Abuaasal et al., 2012; Jones et al., 2012), full characterization of elimination pathways including IE, RTR, and EER is becoming increasingly important. The study clearly demonstrated that 1) IC represents 20–50% of the systemic clearance of apixaban; 2) the overall disposition of apixaban in rats and dogs was governed by a complex and dynamic interplay between absorption, intestinal/renal excretion, and reabsorption in EER and RTR processes; and 3) species-dependent urinary excretion is likely due to different expression of renal efflux transporters. The impact of AC in humans is consistent with IE as an important elimination pathway in addition to metabolism and urinary excretion each accounting for one-third of total clearance in humans. Moreover, apixaban IE and recycling mechanisms of EER and RTR coupled with efflux transporters-limited absorption and its metabolic stability contribute to the overall pharmacokinetic profile of apixaban, including the low peak-to-trough plasma concentration ratio, and help decrease the impacts of intrinsic (e.g., renal and hepatic impairment) and extrinsic (e.g., drug-drug interactions) factors on apixaban disposition.

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Authorship Contributions

Participated in research design: Zhang, He, Rodrigues, Humphreys.
Conducted experiments: Zhang, L. Wang.
Contributed new reagents or analytic tools: Zhang.
Performed data analysis: Zhang, Frost, He, L. Wang, X. Wang, Goosen, Humphreys.
Wrote or contributed to the writing of the manuscript: Zhang, Frost, Rodrigues, X. Wang, Goosen, Humphreys.

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Contributed new reagents or analytic tools: Zhang.

Performed data analysis: Zhang, Frost, He, L. Wang, X. Wang, Goosen, Humphreys.

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