Metabolism and Excretion of Rivaroxaban, an Oral, Direct Factor Xa Inhibitor, in Rats, Dogs, and Humans

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ABSTRACT:

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Rivaroxaban is a novel, oral, direct factor Xa inhibitor for the prevention and treatment of thromboembolic disorders. The objective of this study was to investigate the in vivo metabolism and excretion of rivaroxaban in rats, dogs, and humans. Single doses of [14C]rivaroxaban (3 and 1 mg/kg) were administered to rats (orally/ intravenously) and dogs (orally), respectively. A single oral dose of [¹⁴C]rivaroxaban (10 mg) was administered to healthy human males (n = 4). Plasma and excreta were collected and profiled for radioactivity. Recovery of total radioactivity was high and ≥92% in all species. Unchanged rivaroxaban was the major compound in plasma at all time points investigated, across all species. No major or pharmacologically active circulating metabolites were detected. Rivaroxaban and its metabolites were rapidly excreted; urinary excretion of radioactivity was 25 and 52%, and fecal excretion was 67 and 43% of the dose in rats and dogs, respectively. In humans, 66% of the dose was excreted renally (36% unchanged drug) and 28% in the feces. Radioactivity profiles in excreta were similar across species. Three metabolic pathways were identified: oxidative degradation of the morpholinone moiety (major pathway) and hydrolysis of the central amide bond and of the lactam amide bond in the morpholinone ring (minor pathways). M-1, the main metabolite in excreta of all species, was eliminated via both renal and fecal/biliary routes. In total, 82 to 89% of the dose administered was assigned to unchanged rivaroxaban and its metabolites in the excreta of rats, dogs, and humans.

Rivaroxaban is a novel, oral, direct factor Xa (FXa) inhibitor in advanced clinical development for the prevention and treatment of thromboembolic disorders. More recently, it has received approval in Canada and the European Union for use in the prevention of venous thromboembolism in patients undergoing elective total hip or knee replacement surgery. Rivaroxaban is a selective inhibitor not only of free FXa (K_i 0.4 nM), but also of prothrombinase activity and fibrinassociated FXa activity (Depasse et al., 2005; Perzborn et al., 2005). In vitro, rivaroxaban was shown to inhibit thrombin generation and prolong clotting times (Gerotziafas et al., 2005; Perzborn et al., 2005) and, in vivo, it had potent antithrombotic effects in a variety of animal venous and arterial thrombosis models (Perzborn et al., 2005; Biemond et al., 2007).

Pharmacokinetic (PK) studies of rivaroxaban in rats and dogs have been reported previously (Weinz et al., 2005). These studies demonstrated that rivaroxaban was absorbed rapidly after oral dosing (absolute bioavailability 57-66 and 60-86% in rats and dogs, respectively), had a favorable PK profile with dose proportional increases in area under the concentration-time curve (AUC) and was rapidly excreted via renal and fecal/biliary routes. Rivaroxaban has also been

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shown to demonstrate dose-proportional pharmacokinetics and predictable pharmacodynamics in single (up to 80 mg)- and multipledose studies in healthy subjects and patients with no evidence of accumulation (Kubitza et al., 2005a,b). In addition, rivaroxaban showed high oral bioavailability with rapid absorption and was safe and well tolerated. The present studies were conducted to characterize the metabolism and mass balance of rivaroxaban in vivo in rats, dogs, and humans.

Materials and Methods

Reference Compounds and Chemicals. [14C]Rivaroxaban ([14C]BAY 59-7939) (Fig. 1), with specific activity of 2.69 MBq/mg, enantiomeric purity >99% enantiomeric excess, and UV and radiochemical purity >99%, was synthesized by the Isotope Chemistry Laboratory of Bayer HealthCare AG (Wuppertal, Germany) (Pleiss et al., 2006). Unlabeled rivaroxaban (BAY 59-7939) (Roehrig et al., 2005), dose vehicle for human studies, the internal standard (5-chloro-N-({3,5-dimethyl[4-(3-oxomorpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl}methyl)thiophene-2-carboxamide) and the metabolites M-4 (Pleiss et al., 2006), racemic M-1, M-2, M-7, M-9, the HCl salt of M-15, M-16, M-17, and M-18 were synthesized at Bayer HealthCare AG (Wuppertal, Germany) and Bayer Schering Pharma AG (Berlin, Germany). M-13 (chlorothiophene carboxylic acid) was purchased from Sigma-Aldrich (Steinheim, Germany). Other reagents and solvents were obtained from commercial sources and were of analytical grade or higher purity.

Rat and Dog Studies. All procedures were conducted in accordance with the German Animal Protection Act (Deutsches Tierschutzgesetz). Male Wistar

ABBREVIATIONS: FXa, factor Xa; PK, pharmacokinetic; AUC, area under the concentration-time curve; BAY 59-7939, 5-chloro-N-({(5S)-2-oxo-3-[4-(3-oxo-4-morpholinyl)phenyl]-1,3-oxazolidin-5-yl}methyl)-2-thiophene-carboxamide; BDC, bile duct-cannulated; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; LLOQ, lower limit of quantitation.

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*indicates position of ¹⁴C-label FIG. 1. Chemical structure of [¹⁴C]rivaroxaban.

rats were purchased from Harlan Winkelmann (Borchen, Germany). Female Beagle dogs were obtained from Marshall Farms USA Inc. (North Rose, NY) or Harlan Winkelmann. Animal experiments, from which plasma, urine, bile, and feces specimens were collected for drug metabolism investigations, have been described in detail previously (Weinz et al., 2005).

Human Study. Study design. A single-center, nonrandomized, open-label, nonplacebo-controlled study was conducted in four healthy male subjects, aged 30 to 54 years, with a body mass index of 20 to 27 kg/m². Subjects were excluded from the study if they had any known coagulation disorders (e.g., von Willebrand's disease and hemophilias), conditions associated with an increased risk of bleeding (e.g., periodontitis, hemorrhoids, acute gastroenteritis, and acute peptic ulcer), or known sensitivity to common causes of bleeding. Subjects were also excluded from the study if they had received a radiolabeled substance or had been exposed to significant radiation within the previous 12 months (e.g., serial X-rays, computed tomography scan, or barium meal). The study took place at Inveresk Clinical Research (Riccarton, Edinburgh, UK). It was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation/World Health Organization Good Clinical Practice standards guidelines and with the approval of the Independent Ethics Review Committee of Inveresk Clinical Research and the Department of Health (UK) Administration of Radioactive Substances Advisory Committee. All subjects provided written informed consent. Subjects entered the clinic 1 day before drug administration and were discharged 7 days after drug administration. Subjects fasted overnight before administration of study drug and received their next meal 4 h postdose.

Dosage form and administration. Subjects received a single, oral dose of a solution of approximately 10 mg of rivaroxaban, containing [¹⁴C]rivaroxaban plus unlabeled rivaroxaban with a mean radioactive dose of 47.5 μ Ci (1.76 MBq). The solution was administered with 240 ml of tap water. Doses were given orally, because this is the route of administration in the clinical setting. The actual radioactive dose received by each subject was determined with reference to the dose concentration, the weight of the dose administered, and the specific activity of [¹⁴C]rivaroxaban in the formulated dose.

Specimen collection and preparation. Blood samples (approximately 10 ml) were collected in heparinized vials at prespecified time points (Table 1) from an in situ venous cannula or by venipuncture. Samples were centrifuged at 3000 rpm for 10 min at approximately 4°C to obtain plasma, which was stored

at approximately -20° C until analysis. Urine was collected over prespecified time intervals (Table 1), weighed, and stored at -20° C until analysis. Feces were collected daily (Table 1), weighed, and homogenized after dilution with an amount of water corresponding to their weight.

Measurement of Radioactivity. Total radioactivity in liquid samples [plasma, urine and bile (BDC rats only)] from all species was determined by liquid scintillation counting using a Tri-Carb 2500A liquid scintillation spectrometer (Canberra Packard, Groningen, The Netherlands). Samples were made up to 1 ml with distilled water, if necessary, and prepared for radioanalysis by the addition of Ultima Gold scintillation cocktail. Solid samples (feces) were homogenized, lyophilized, homogenized again, and combusted using the Canberra Packard System 387 (Oxidizer Tri-Carb 307 and Robot System 80). The formed ¹⁴CO₂ was trapped in Carbo-Sorb and topped up with scintillation cocktail (Permafluor E⁺) before radioanalysis.

Metabolite Profiling. Metabolite patterns in plasma, urine, and feces from all species and in bile from BDC rats were determined using high-performance liquid chromatography (HPLC) with on-line radioactivity detection (Ramona 5; Raytest GmbH, Straubenhardt, Germany) or off-line radioactivity detection by liquid scintillation counting (Wallac 1450 Microbeta Plus scintillation counter; Wallac, Freiburg, Germany) (Lang et al., 2009). Plasma samples (1 or 2 ml for human samples with a radioactivity concentration <1000 dpm/ml) were precipitated by adding acetonitrile (2 or 4 ml), followed by centrifugation. The supernatant was removed, and the residue was washed with a further 1 ml of acetonitrile. Urine samples were either analyzed by HPLC directly, without pretreatment (for those samples containing high amounts of radioactivity), or extracted using Waters Oasis cartridges (Waters GmBH, Eschborn, Germany) before HPLC analysis. For urine extraction, a 3-ml urine sample was diluted with 1 ml of water and added to a preconditioned Waters Oasis cartridge (2 ml of methanol followed by 2 ml of water). Each cartridge was washed with 3.5 ml of water, and the extracts were eluted with 1.5 ml of acetonitrile. Feces were extracted by adding approximately 2 ml of acetonitrile to approximately 1 ml of feces suspension. After vortexing and centrifugation, the supernatant was removed, and the residue washed with 1 to 2 ml of acetonitrile (depending on the sample weight). For plasma, urine, and feces samples, extracts were combined for each time interval and concentrated by evaporation, and an aliquot was removed for HPLC analysis. Bile samples from BDC rats were directly analyzed by HPLC.

Rivaroxaban and its metabolites were separated on a Nucleosil 100-5 C8 HD column (125 \times 2 mm; Macherey-Nagel, Düren, Germany) with a guard column (8 \times 3 mm). For better separation and assignment of closely eluting metabolites M-2, M-3, and M-8/M-9, a Nucleosil 100-5 CN column with a guard column (70 \times 2 mm) was used. Separation of M-8/M-9 could not be achieved, and these metabolites were thus balanced together.

Metabolite Identification and Quantitation. Metabolites were identified by chromatographic characteristics and liquid chromatography (LC)/mass spectrometry (MS) analysis. In addition, some metabolites of rivaroxaban were isolated by preparative HPLC from in vivo samples (rat urine, bile, and feces and human urine); after purification, the structure was confirmed by ¹H NMR spectroscopy. The metabolic fate of the unlabeled (*S*)-oxamine moiety of

	Summary of [1	⁴ C]rivaroxaban doses, routes of ad	ministration and timing of	biological sample	e collection in rats, dogs, and humans
Species	Route	Single Dose	Number	Matrix	Sampling Time
Rat	p.o. <i>a</i>	3 mg/kg (2.69 MBq/mg)	3 per time point	Plasma	1, 2, 4, 8 h
				Urine	0-8, 8-24 h, daily until 168 h
				Feces	Daily until 168 h
	i.v. ^b	3 mg/kg (2.69 MBq/mg)	5	Urine	0-4, 4-8, 8-24 h
				Bile	0–4, 4–8, 8–24 h
				Feces	0–24 h
Dog	p.o.	1 mg/kg (2.69 MBq/mg)	3	Plasma	0.25, 0.5, 1, 2, 4, 8, 10 h
	<u>^</u>			Urine	Daily until 168 h
				Feces	Daily until 168 h
Human	p.o.	$10 \text{ mg} (1.76 \text{ MBq})^c$	4	Plasma	0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8,
	*				12, 15, 24 h
				Urine	0-4, 4-8, 8-12, 12-24 h, daily until 168 h
				Feces	Daily until 168 h
					-

TABLE 1

^a Intact rats.

^b Bile duct-cannulated rats.

^c Mean actual dose received by human subjects.



FIG. 2. Plasma concentration-time profiles of total radioactivity and unchanged rivaroxaban after a single oral administration of 10 mg of [¹⁴C]rivaroxaban to healthy human subjects (n = 4). Data are presented as geometric means \pm S.D. (semilogarithmic scale).

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rivaroxaban was investigated using LC-MS/MS analysis on an API 3000 mass spectrometer. HPLC chromatography was performed with an Agilent Zorbax SB-C18 column (30×2.1 mm), and 10 mM ammonium formate (pH 4) and acetonitrile as mobile phases. Metabolite M-4 was analyzed in electrospray ionization negative mode; all other metabolites were analyzed in electrospray ionization positive mode, with an internal standard used in previous studies (Weinz et al., 2005). Appropriate and selective MS/MS transitions were evaluated for all analytes. The lower limit of quantitation (LLOQ) of the method ranged from 5 to 50 ng/ml for all analytes, and the linear range was up to at least 1000 ng/ml. For calibration purposes, control human plasma and urine samples were spiked with different concentrations of all analytes in a range of 1 to 1000 ng/ml. Acetonitrile containing the internal standard was added to precipitate proteins in all plasma samples. For chromatographic reasons the supernatants were diluted with water and then analyzed using LC-MS/MS. Details of metabolite structure elucidation and in vitro studies are reported in the companion article (Lang et al., 2009).

Pharmacokinetic Analysis. Mean plasma concentration versus time data for total radioactivity, unchanged rivaroxaban, and metabolite M-1 were analyzed noncompartmentally (Yamaoka et al., 1978) using software developed in-house (KINCALC, version 2.50.02, 2001).

Results

Pharmacokinetic Evaluation. *Humans.* Rivaroxaban was well tolerated, and no serious adverse event was observed during the course of the study. Extraction recovery of radioactivity in plasma, urine, and feces was in most cases >90% and absolute LLOQ was approximately 5 ng of drug equivalent.

The plasma concentration-time profiles of total radioactivity and unchanged rivaroxaban after oral administration of [¹⁴C]rivaroxaban to healthy human subjects are shown in Fig. 2. Total radioactivity and unchanged rivaroxaban reached the maximum plasma concentration (C_{max}) 30 min postdose, and both were eliminated rapidly and with a parallel time course from plasma; at time points later than 24 h after drug administration; the concentrations were below the LLOQ. The AUC of unchanged rivaroxaban accounted for approximately 89% of the AUC of total radioactivity in plasma, and the PK parameters for unchanged rivaroxaban were very similar to those reported for the 10-mg dose in the single-dose escalation study (Kubitza et al., 2005a).

Rats and dogs. The plasma concentration-time profiles of total radioactivity and unchanged rivaroxaban and PK parameters after oral administration of [¹⁴C]rivaroxaban to rats and dogs were published elsewhere (Weinz et al., 2005).

Excretion of Radioactivity in Urine and Bile/Feces. *Humans.* The recovery of total radioactivity in excreta (urine and feces) 7 days postdose accounted for 94% of the dose administered (Table 2; Fig. 3). Radioactivity was excreted predominantly in urine, with an average of 66% of the administered radioactive dose being excreted after 7 days; approximately 28% was excreted in the feces (Table 2; Fig. 3). A large proportion of the dose (mean 68%) was recovered in urine and feces within the first 24 h.

Rats and dogs. In the oral studies in rats and dogs, the recovery of total radioactivity in excreta 7 days postdose accounted for approximately 92 and 97% of the dose, respectively (Table 2) (Weinz et al., 2005). In the intravenous study in BDC rats, recovery was approximately 92% after 1 day. Excretion of radioactivity in rats occurred mainly via the biliary/fecal route, with 61 to 67% of dose after oral or intravenous administration of [¹⁴C]rivaroxaban. After intravenous administration to BDC rats, approximately 48% was excreted via the biliary and approximately 13% via the extrabiliary route (Table 2). Renal excretion accounted for 25 to 30% in rats. In dogs, both excretion pathways were of similar importance, with approximately 52% renal and 43% fecal excretion (Table 2).

Metabolite Profiles in Plasma. *Humans*. Unchanged rivaroxaban was identified as the main compound in human plasma at all time points investigated after oral administration of 10 mg of $[^{14}C]$ rivar-

TABLE 2

Excretion of total radioactivity after single administration of $[^{14}C]$ rivaroxaban to rats, dogs, and humans

		Sample	Radioactivity							
Species	Route	Collection Time	Urine	Bile	Feces	Cage Wash	Recovery			
		days			% dos	?				
Rat	p.o. ^{<i>a</i>}	7	24.7		66.9		91.6			
Rat	i.v. ^b	1	30.3	48.4	12.9		91.6			
Dog	p.o.	7	52.2		42.8	2.0	97.0			
Human	p.o.	7	65.7		28.0		93.7			

^{*a*} Intact rats. ^{*b*} Bile duct-cannulated rats



FIG. 3. Cumulative excretion of radioactivity (percentage of administered dose) in urine and feces after a single oral dose of [¹⁴C]rivaroxaban to healthy human subjects (n = 4). Data are presented as arithmetic means \pm S.D.

oxaban, accounting for 89% of the AUC from 0 to the terminal time point (AUC_{0-tn}) of total radioactivity in all four subjects (Fig. 4). A few minor metabolites were detected; among these were M-1, M-4, M-5, M-7, and M-8/M-9. The most prominent metabolite (M-1) accounted for only 3% of the AUC_{0-tn} of total radioactivity on average. The elucidation of the structure of these metabolites is published in the companion article (Lang et al., 2009). More than 95% of the total radioactivity in plasma could be attributed to unchanged rivaroxaban and known metabolites of rivaroxaban.

The amide hydrolysis of [¹⁴C]rivaroxaban, leading to M-13 and subsequently to M-4, was of special interest, because the radioactive ¹⁴C label is located on the carbonyl moiety adjacent to the chlorothiophene moiety. Therefore, only M-4 and not the unlabeled part of the compound is considered in ¹⁴C-mass balance studies of rivaroxaban. The mechanism of cleavage and the metabolic fate of the unlabeled part of the molecule have been elucidated in vitro (Lang et al., 2009). To investigate the importance of these unlabeled metabolites in man in vivo, human plasma samples were analyzed using a sensitive HPLC-MS/MS assay for the quantitation of M-15, M-16, M-17, and M-18 against M-4 (Lang et al., 2009). None of the unlabeled metabolites was detected in human plasma samples, and only trace amounts of M-4 were found. Overall, no major circulating metabolite of rivaroxaban was found in human plasma.

Rats and dogs. Unchanged rivaroxaban was identified as the main compound in rat and dog plasma at all time points investigated after oral administration of [¹⁴C]rivaroxaban (Fig. 4). The AUC of unchanged rivaroxaban accounted for 83 and 71% of the AUC of total radioactivity in rats and dogs, respectively. Few minor metabolites were detected in plasma; of these, the most prominent metabolite, M-1, accounted for only 6 and 5% of the AUC of total radioactivity in rat and dog plasma, respectively. A few minor metabolites were observed in both species, among which M-2 and M-8/M-9 were identified in rat plasma and M-2, M-6, and M-7 in dog plasma. Total radioactivity, unchanged rivaroxaban, and metabolite M-1 were eliminated rapidly after oral administration. Similarly to humans, no major circulating metabolite was found in the plasma of rats and dogs.

Metabolite Profiles in Urine and Bile/Feces. *Humans.* After oral administration of [¹⁴C]rivaroxaban to healthy human subjects, metab-

olite M-1 was identified as the main metabolite in excreta. M-1 was eliminated via renal and fecal/biliary routes, in total accounting for 22% of the dose. Metabolites M-4 and M-7 were found in almost equal amounts of 7 to 8% of the dose (Tables 3 and 4; Fig. 5). M-4 was eliminated exclusively via the renal route (Table 3), whereas M-7 was eliminated via renal and fecal routes. Few other minor metabolites were observed in excreta. Metabolic profiles in urine and feces were qualitatively similar between the different sampling intervals. However, unchanged rivaroxaban was the major component in urine, accounting for 36% of the total dose; approximately 7% of the dose was excreted fecally. In total, approximately 43% of the dose was excreted as unchanged drug in humans. Overall, 89% of the radioactive dose administered could be assigned to unchanged rivaroxaban and known metabolites of rivaroxaban in human excreta. Similarly to human plasma, human urine fractions were analyzed using a sensitive HPLC-MS/MS assay for the quantification of the unlabeled metabolites M-15, M-16, M-17, and M-18, against M-4 (Lang et al., 2009). Only metabolites M-15, M-17, and M-18 were found and quantified in human urine. A mass balance of these unlabeled metabolites against the radiolabeled counterpart M-4 was established, for which M-4 was quantified either by radioanalysis or by specific LC-MS/MS analysis. The LC-MS/MS quantification of M-4 was in the range of 93 to 112%, compared with the data quantified using radioactivity (Table 5). Furthermore, the mass balance of the unlabeled metabolites M-15, M-17, and M-18 against the labeled counterpart M-4 in human urine samples was in the range of 81 to 113% (Table 5). These in vivo data confirmed the proposed in vitro pathway of a hydrolytic cleavage of rivaroxaban and provided strong evidence that metabolites M-4 and M-15/M-17/M-18 represent the endpoints of this metabolic pathway.

Rats and dogs. Two major metabolites, M-1 and M-4, and few minor metabolites were observed in excreta after intravenous administration of [¹⁴C]rivaroxaban to BDC rats and after oral administration to dogs (Tables 3 and 4). M-1 was identified as the main metabolite in rats and dogs and was eliminated via the renal and fecal/biliary routes (Tables 3 and 4). As in humans, M-4 was excreted exclusively via the renal route (Table 3). Metabolic patterns in excreta were qualitatively similar across different sampling intervals in both species.

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FIG. 4. Radioactivity profile of plasma 1 h after oral administration of $[^{14}C]$ rivaroxaban to a male Wistar rat (A), a female Beagle dog (B), and a healthy human male (C).

TABLE 3

Metabolite balances in urine, bile, and feces (selected metabolites) after administration of $[^{14}C]$ rivaroxaban to rats, dogs, and humans in time interval analyzed

Sample	Species	Time	Route	%Dose in Time Interval	Radioactivity								
					Rivaroxaban	M-1	M-2	M-4	M-5	M-6	M-7	M-8/M-9	Total ^a
		days							% dose				
Urine	Rat	0-2	p.o. ^b	23.5	5.2	9.1	4.1	3.2	0.1			0.7	22.4
	Rat	0-1	i.v. ^c	30.3	8.1	11.9	3.4	5.4				1.0	29.8
	Dog	0-5	p.o.	52.0	7.9	8.5	7.8	15.7	0.6	0.5	0.9	1.9	43.8
	Human	0-2	p.o.	65.4	36.2	13.1	0.7	7.6	0.9	0.3	3.7	2.1	64.6
Bile	Rat	0-1	i.v. ^c	48.4		44.4							44.4
Feces	Rat	0-1	i.v. ^{<i>c</i>}	12.9	2.9	5.5	2.5		1.9	< 0.1			12.9
	Dog	0-5	p.o.	42.5	1.1	26.2	2.8		2.1	2.1	2.9	0.7	37.9
	Human	0-4	p.o.	26.4	7.3	8.9	1.1		1.6	0.9	2.9	1.6	24.3

^a Unchanged rivaroxaban and known metabolites

^b Intact rats.

^c Bile duct-cannulated rats.

In urine, unchanged rivaroxaban and known metabolites of rivaroxaban accounted for approximately 25 and 30% of the administered radioactive dose in intact and BDC rats, respectively, and for 44% of the dose in dogs (0–5 days postdose). M-1 was the major metabolite in rat urine (12% of the dose), and M-4 was the major metabolite in dog urine (16% of the dose) (Table 3).

Extensive metabolism of rivaroxaban was observed in the excreta of BDC rats after i.v. administration (Tables 3 and 4). Unchanged rivaroxaban accounted for only 11% of the administered radioactive dose, but M-1 accounted for approximately 62%. Metabolite M-1 was the only metabolite detected in bile, representing 44% of the dose (Table 3). Overall, 87 and 82% of the administered radioactive dose could be assigned to unchanged rivaroxaban and known metabolites of rivaroxaban in rats and dogs, respectively.

Discussion

The studies summarized in this article focus on the metabolism and excretion of radiolabeled rivaroxaban ([¹⁴C]rivaroxaban) after administration of single intravenous and oral doses to rats and after administration of a single oral dose to dogs and humans.

Unchanged rivaroxaban accounted for the majority of total radioactivity in human plasma after oral administration of 10 mg of $[^{14}C]$ rivaroxaban, reflecting a minimal presence of metabolites. Accordingly, the plasma concentration-time curve for unchanged rivaroxaban was very similar to that for total radioactivity, showing that the pharmacological effects of rivaroxaban are solely due to unchanged drug. Rivaroxaban was absorbed rapidly after oral administration to humans (t_{max} 30 min) and subsequently was eliminated rapidly from plasma to give concentrations below the LLOQ at time points later than 24 h after drug administration. This result suggests that the pharmacodynamic effects of rivaroxaban in humans would be initiated rapidly after oral administration and would cease rapidly after treatment was terminated (Kubitza et al., 2005a,b). Unchanged rivaroxaban was also identified as the main compound in the plasma of rats and dogs. In all three species, no major radiolabeled circulating metabolite was detected in plasma, and the most prominent of the minor metabolites, M-1, accounted for only a very small proportion of total radioactivity in plasma (approximately 3–6% across all species). In addition, none of the unlabeled metabolites M-15, M-16, M-17, and M-18, derived from the hydrolysis of the amide bond at the chloro-thiophene moiety, was detected in human plasma.

The PK characteristics of rivaroxaban, derived from this mass balance study, were consistent with the findings of previous phase I studies investigating single and multiple doses of rivaroxaban in healthy human subjects (Kubitza et al., 2005a,b).

[¹⁴C]Rivaroxaban-associated radioactivity was excreted rapidly in humans. Excretion occurred predominantly via the renal route (66%) and to a lesser extent via fecal/biliary routes (28%), with 36% of rivaroxaban excreted unchanged in the urine and 7% excreted unchanged in feces. Rapid, dual-mode excretion of rivaroxaban was also observed in rats and dogs, although the contribution of each excretion route to the total excretion of radioactivity differed from that in humans. In dogs, renal and fecal/biliary pathways were of approximately similar importance; in rats, excretion occurred mainly via the fecal/biliary routes. The recovery of approximately 13% of radioactivity in feces after intravenous administration to BDC rats indicates that [¹⁴C]rivaroxaban-associated radioactivity undergoes extrabiliary excretion to a certain extent.

The administered radioactive doses were almost completely recovered (94%) in the excreta of humans 7 days after administration. Likewise, the recovery of radioactivity in excreta 24 h after intravenous administration to BDC rats, 48 h after intravenous or oral administration to intact rats, and 7 days after oral administration to dogs, accounted for the majority of the administered radioactive dose (92 and 97%, respectively). The metabolic pathways of rivaroxaban in humans were similar to those in rats and dogs and to those observed in the in vitro metabolism studies using liver microsomes and hepatocytes from different species, including man (Lang et al., 2009).

TABLE 4

Summarized metabolite balances in excreta (selected metabolites) after administration of $[^{I4}C]$ rivaroxaban to rats, dogs, and humans

Species	D		Radioactivity								
	Route	Rivaroxaban	M-1	M-2	M-4	M-5	M-6	M-7 M-8/M-9	Total ^a		
						% dose					
Rat	i.v. ^b	11.0	61.8	5.9	5.4	1.9	< 0.1		1.0	87.1	
Dog	p.o.	9.0	34.7	10.6	15.7	2.7	2.6	3.8	2.6	81.7	
Human	p.o.	43.5	22.0	1.8	7.6	2.5	1.2	6.6	3.7	88.9	

^a Unchanged rivaroxaban and known metabolites

^b Bile duct-cannulated rats.



Fig. 5. Radioactivity profile of human urine fraction (0–4 h) (A) and human fecal extract (2–3 days) (B) after oral administration of [14 C]rivaroxaban to one subject.

A high mass balance of known metabolites was achieved in the in vivo metabolism studies. The majority of the administered radioactive dose could be assigned to rivaroxaban and all known metabolites of rivaroxaban in rat, dog, and human excreta after intravenous or oral administration of [¹⁴C]rivaroxaban. M-1 was identified as the major metabolite in human excreta and was eliminated via renal and fecal/ biliary routes. Metabolites M-2, M-4, and M-7 were identified as less

prevalent metabolites. M-4 was excreted exclusively via the renal route, whereas M-2 and M-7 were excreted through both the renal and the fecal/biliary pathways. The urinary and fecal/biliary metabolite profiles in rats and dogs were qualitatively similar to those in humans, although M-4 was the major metabolite in urine of dogs (rather than M-1), and M-7 was found in dogs but not in rats.

Structural studies revealed that the metabolite M-1 was produced

Summarized data for the mass balance calculation in urine of the sum of unlabeled metabolites M-15, M-17, and M-18 against counterpart M-4 in healthy volunteers after administration of 10 mg of $[1^{4}C]$ rivaroxaban

Volunteer No.	M-15	M-17	M-18	Total M-15, M-17, and M-18		M-4	Total M-15, M-17, and M-18 vs. M-4		
					LC-MS	Radiodetection	LC-MS	Radiodetection	
				μmol				%	
001M 002M 003M	0.14 0.22 0.19	0.26 0.29 0.47	1.22 0.81 1.80	1.62 1.32 2.46	2.01 1.29 2.18	1.79 1.30 2.34	81 102 113	91 102 105	
004M	0.21	0.24	1.05	1.51	1.65	1.47	91	103	



FIG. 6. Proposed metabolic pathways of rivaroxaban in vivo in rats, dogs, and humans.

through oxidative degradation of the morpholinone moiety of rivaroxaban (forming M-2), followed by oxidative cleavage of the ring (Lang et al., 2009). In addition, the minor metabolites M-5 and M-6 are also products of the oxidative degradation of the morpholinone ring. Metabolite M-4 was produced by hydrolysis of the amide bond of rivaroxaban, with subsequent conjugation of the 5-chloro-2-thiophene-carboxylic acid (M-13) with glycine. Metabolite M-7 results from the hydrolytic cleavage of the lactam amide bond in the morpholinone moiety. It can be concluded that the metabolism of rivaroxaban occurs via two major pathways: oxidative degradation of the morpholinone moiety and hydrolysis of the different amide bonds.

In humans, rivaroxaban is eliminated via three pathways: approximately 43% of the dose is excreted as unchanged drug via urine (36%) and feces (7%, representing nonabsorbed or extrabiliarily excreted material); approximately 14% of the dose (M-4 and M-7) is eliminated via hydrolytic cleavage of the two different amide bonds; and approximately 32% of the dose is eliminated via oxidative pathways. The proposed scheme for the metabolism of rivaroxaban, based on structures of all known metabolites and metabolite profiles, is depicted in Fig. 6. The renal excretion of one-third (36%) of the dose as unchanged drug suggests the involvement of transport proteins. In vitro studies showed rivaroxaban to be a substrate for P-glycoprotein and breast cancer resistance protein. Details of these studies will be published elsewhere (M. Gnoth, S. Sandmann, U. Muenster, and U. Buetehorn, manuscript in preparation).

In summary, the present study demonstrated high species similarity

in the metabolism of rivaroxaban in humans, rats, and dogs, with almost all administered dose assigned to known structures. Comparison of the in vivo data presented here with the in vitro data published in a companion article (Lang et al., 2009) indicates very good in vitro/in vivo correlation. Reactive metabolites were not found in either in vitro or in vivo studies.

After rapid absorption, rivaroxaban is metabolized via different types of biotransformation reactions, and the drug and metabolites are rapidly eliminated via renal and biliary/fecal routes. No major circulating active metabolites were observed. In humans, a dual mode of elimination was observed, with almost half of the dose excreted as unchanged drug (in the urine and feces) and the other half excreted after metabolic biotransformation.

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