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Tissue distribution and elimination of C-14 apixaban in rats

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Abbreviations used: AUC, area under the plasma concentration-time curve; BCRP, breast cancer resistance protein; BLQ, below limit of quantitation; LSC, liquid scintillation counting; LC/MS, liquid chromatography/mass spectrometry; MS/MS, tandem mass spectrometry; Mrp, multidrug resistance protein; LLOQ, low limit of quantitation; P-gp, P-glycoprotein; PK, pharmacokinetics; QWBA, Whole-body autoradiography; SD, Sprague-Dawley rats.

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ABSTRACT

Apixaban, a potent and highly selective factor Xa inhibitor, is currently under development for treatment of arterial and venous thrombotic diseases. The distribution, metabolism, and elimination of C-14 apixaban were investigated in male, female, pregnant and lactating rats following single oral doses. Tissue distribution of radioactivity in rats was measured using quantitative whole-body autoradiography. Following single oral administration, radioactivity distributed quickly in rats with C_{max} at 1 h for most tissues. The elimination $t_{1/2}$ of radioactivity in blood was 1.7 to 4.2 h. The blood AUC of radioactivity was similar between male and female rats and was slightly higher in pregnant and lower in lactating rats. The radioactivity concentration in tissues involved in elimination was greater than blood with the highest concentration in gastrointestinal tracts, liver, urinary bladder/contents, and lowest level in brains. In pregnant rats, the whole-body autoradiogram showed that low levels of radioactivity were present in fetal blood, liver, and kidney, and were much lower than the radioactivity in respective maternal organs. Fecal route was the major (74% of dose) and urinary was minor pathway (14%) for apixaban elimination. Following single oral doses of C-14 apixaban to lactating rats, apixaban exhibited extensive lacteal excretion with apixaban as the major component. In summary, tissue distribution of apixaban in rats was extensive, but with limited transfer to fetal and brain tissues and extensive secretion into rat milk with parent drug as the major component. Milk excretion could account for 10% of apixaban dose, which was comparable to urinary elimination in rats. Tissue distribution and drug excretion of apixaban are consistent with a moderately permeable drug that is a substrate for P-gp and BCRP efflux transporters.

INTRODUCTION

Factor Xa is a key serine protease in the coagulation cascade and is a promising target enzyme for new therapeutic agents to treat and prevent arterial and venous thrombosis (Kaiser, 2002; Samama, 2002; Walenga et al., 2003). In particular, factor Xa plays a critical role in blood coagulation, serving as the juncture between the extrinsic (tissue factor initiated) and intrinsic (surface activation and amplification) systems (Mann et al., 2003). Apixaban is an oral, potent, reversible, selective and direct factor Xa (FXa) inhibitor, which inhibits both free and prothrombinase-bound FXa activity, and shows considerable efficacy in the prevention of arterial and venous thrombosis at doses that preserved hemostasis in rabbits (Pinto et al., 2007; Wong et al., 2008). Apixaban is also effective and safe for the prevention and treatment of venous thrombosis in humans (Lassen et al., 2007; Buller et al., 2008; APPRAISE Steering Committee and Investigators, 2009). Apixaban was well absorbed in rats, dogs, and humans (bioavailability of 34-80%, respectively). The mean volume distribution of apixaban was 0.31, 0.2-0.29, and 0.3 L/kg in rats, dogs, and humans, respectively. The systemic clearance of apixaban was low, representing<10%, <3%, and 5% of hepatic blood flows in rats, dogs, and humans, respectively (Shantsila and Lip, 2008). Apixaban was metabolized by multiple pathways in animals and humans (Zhang et al., 2009) and the fecal route was a major elimination pathway, accounting for >54% of dose in animals and >46% in humans. The urinary elimination accounted for <15% in animals and 25-28% of dose in humans (Zhang et al., 2009). In this study, the distribution, elimination, and milk excretion properties following single oral doses of [^{14}C]apixaban in male, female, pregnant, and lactating rats were investigated.

MATERIALS AND METHODS

Materials. [^{14}C]Apixaban (specific activity: 20.1 μCi/mg and radiochemical purity >99.8%) was synthesized at Bristol-Myers Squibb (Princeton, NJ) (Zhang et al., 2009). All organic solvents and water were of HPLC grade.

Dose preparation. The oral dosing formulation was prepared on the day of dosing by addition of [^{14}C]apixaban to a 0.5% Tween 80 in Labrafil® (w/w) vehicle. For tissue distribution study, the final drug concentration and radioactivity of [^{14}C]apixaban in dosing solution was 1.0 mg/mL and 20 μCi/mL, respectively; For milk excretion study, the final drug concentration and radioactivity of [^{14}C]apixaban in dosing solution was 5.0 mg/mL and 20 μCi/mL, respectively. The concentration and homogeneity of [^{14}C]apixaban were verified prior to and after dosing by counting triplicate aliquots of the formulation using liquid scintillation counting (LSC). The mean pre-dose radioactivity concentration was used in calculation of the amount of radioactivity administered to each animal.

Animal preparation and dosing. Before the study initiation, the study protocol was approved by the Institutional Animal care and Use Committee. All animal housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). Sprague Dawley (SD) rats were purchased from Harlan Laboratories (Indianapolis, Indiana) or Hilltop Laboratories (Scottdale, PA). Each animal was weighed, randomized, assigned a permanent identification number, and identified with a tail mark. During the acclimation period (at least two days prior to study initiation), the animals were housed in individual, suspended, stainless steel wire mesh cages and food (Certified Rodent Diet #5002, PMI Nutrition International) and water were provided *ad libitum*, except when animals were fasted

overnight (at least 12 h) prior to, and through 4 h following dose administration. The animal room was controlled to maintain a temperature of 75ºF, and 70% relative humidity, with a 12 h light/12 h dark cycle. The light cycles may have occasionally been interrupted to perform study procedures. Animals were placed in individual plastic shoe-box cages with raised wire flooring and bedding and housed in these cages throughout the study period. The body weight of each rat was determined on the day before dosing. The doses administered were calculated based on the body weight of each rat. The formulated [^{14}C]apixaban was 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. The amount of dose administered to each rat 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.

Tissue distribution study. Eight male SD rats (Group 1, body weight of 225-252 g), 8 female SD rats (Group 2, body weight of 206-213 g), and 6 pregnant female SD rats (Group 3, body weight of 270-319 g) were used for tissue distribution study. Each rat received a single oral dose of [^{14}C]apixaban at 5 mg/kg (100 μCi/kg). After dosing, rats were returned to their home cage and cage-side observations were performed on the day of dosing and at least daily for the remainder of the study. One male rat per time point from Group 1 and one female rat per time point from Group 2 were euthanized at 0.5, 1, 4, 8, 24, 72, 96, and 168 h post-dose. One pregnant female rat was euthanized at 0.5, 1, 4, 8, 24, and 48 h. Blood sample (4 to 10 mL) from each rat was collected into tubes containing K_3EDTA anticoagulant and was placed immediately on wet ice, and then centrifuged at 1300g for 10 min to obtain plasma and blood cells. Animals were euthanized and then each

carcass was frozen in a hexane/dry ice bath for at least 15 min. All carcasses were stored at -20°C prior to preparation for tissue distribution by QWBA.

Milk excretion study. Nineteen female SD rats were used for this study and each received a single oral dose of [^{14}C]apixaban at 5 mg/kg (108 μCi/kg). Milk was collected from one rat predose and 3 rats/time point at 0.5, 1, 2, 6, 12, and 24 h postdose. Rats were given a subcutaneous injection (0.2 mL) of oxytocin 15 min prior to milk collection to stimulate lactation. Rats were anesthetized before the start of milk collection, and 1 mL milk was collected from each rat using a specially constructed milking machine. The weight of each milk sample was recorded and samples were placed immediately on wet ice or refrigerated until aliquoted for radioanalysis. Following milk collection, rats were sacrificed by cardiac puncture under isoflurane anesthesia and 4 to 10 mL blood sample was collected into tubes containing K_3EDTA anticoagulant. Blood was placed immediately on wet ice, and then centrifuged at 1300g for 10 min to obtain plasma.

Elimination study. Three male SD rats (body weight approximately 300 g) were used for mass balance study. Each rat received a single oral dose of [^{14}C]apixaban at 30 mg/kg (150 μCi/kg). Urine and feces were collected from each rat over the time interval of 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 h postdose.

Whole-body autoradiography. QWBA assays were performed essentially as described previously (Solon et al., 2002). Briefly, each frozen rat carcass was embedded in a 2% carboxymethylcellulose matrix with a microtome stage at -20°C. Three quality control standards (QC) of [^{14}C]glucose at 0.05 μCi/g were placed into the frozen blocks prior to sectioning, and were used for section thickness quality control. Whole-body sections at

40 µm thick were taken in the sagittal plane, and captured on adhesive tape (Scotch Tape No. 8210, 3M Ltd., St. Paul, MN, USA). Sections at various levels in the whole body were collected to include the major tissues, organs, and biological fluids, the whole-body sections were dried in the cryomicrotome at -20°C for at least 48 h. Once dried, a set of sections for each rat was mounted on a cardboard backing, covered with a thin plastic wrap, and exposed along with calibration standards of [^{14}C]glucose at 10 different concentrations (0.001 to 6.5 µCi/g) to a ^{14}C-sensitive phosphor imaging plate (Fuji Biomedical, Stamford, CT). The imaging plates and sections were placed in light-tight exposure cassettes and were kept in a copper-lined lead, radiation safe chamber for a 4-day exposure at room temperature. After exposure, the imaging plates were scanned using the Typhoon 9410 image acquisition system (GE Healthcare/Molecular Dynamics, Sunnyvale, CA, USA) and the whole-body images stored in a computer. Radioactivity concentration of tissues was quantified using the MCID image analysis software (v. 7.0, GE Healthcare/Imaging Research, Inc., St. Catherines, Ontario, Canada) based on the calibration standards of [^{14}C]glucose. The concentrations of radioactivity were expressed as the µg equivalents of apixaban per gram sample (µg eq/g). An upper (ULOQ) and lower (LLOQ) limit of quantification was applied to the data. The ULOQ and LLOQ were determined by using the radioactive concentration of the highest and lowest calibration standards divided by the specific activity of the test article in formulation.

Radioactivity determination. Radioactivity in samples of plasma, blood, milk, urine or feces was determined using a Packard Tri-Carb® model 2250CA liquid scintillation counter (LSC) and recovery of radioactivity in urine and feces was calculated. Fifty to one hundred microlitre of each plasma, blood, milk, or urine sample was used for radioactivity

determination in duplicate by adding 15 mL Ecolite™ liquid scintillation cocktail and was counted for 10 min. Individual homogenized fecal samples (0.2 g each) were added with 1 mL Soluene-350 and incubated overnight with gentle shaking in a 60°C water bath. After cooling, 0.2 mL of 30% hydrogen peroxide and 15 mL Ecolite™ were added to each sample, mixed well, and then was counted for 10 min in LSC.

Metabolite profile. Metabolites in rat plasma, milk, urine, and fecal samples were profiled as described previously (Zhang et. al., 2009; Raghavan et al., 2009). Briefly, pooled plasma, milk, and feces samples were extracted in duplicate by addition of 4 mL of acetonitrile/methanol (1:1, v/v) to 1 mL of each sample while the sample was mixed on a vortex mixer. After centrifugation at 2000*g* for 1 h, each supernatant fraction was removed and saved. The precipitate was resuspended in 2 mL of acetonitrile and 1 mL of methanol. Following centrifugation of the mixture for 30 min at 2000*g*, the supernatant fraction was removed and combined with the first supernatant. The combined supernatant fraction was evaporated to dryness under nitrogen and reconstituted in 0.15 mL of acetonitrile and 0.05 mL of methanol. Following centrifugation at 2000*g* for 5 min, a portion of 100 μL supernatant was injected into the HPLC for metabolite profiling and identification. Pooled urine sample (1 mL) was centrifuged at 2000*g* for 5 min, a portion of 100 μL supernatant was injected into the HPLC for metabolite profiling and identification. The HPLC system was an Agilent 1100 series system equipped with two pumps, an autoinjector, a UV detector, and an ACE® 3 C18 column (4.6 mm x 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 employed was as follows: Solvent B started at 5%, then linearly increased to 20% at 5 min, to 30% at 50 min, to 35% at 55 min, to 90% at 65 min, held at 90% for 2 min, and

then decreased to 5% at 69 min. The HPLC effluent (0.7 mL/min) was collected into Deepwell LumaPlate™-96 plates (PerkinElmer Life and Analytical Sciences, Shelton, CT) at 0.25 min intervals for 75 min with a Gilson Model 204 fraction collector (Gilson Medical Electronics, Middleton, WI). The plates were dried with a Savant Speed-Vac System (Global Medical Instrumentation, Inc., Ramsey, MN) and counted for 10 min per well with a TopCount analyzer (PerkinElmer Life and Analytical Sciences, Shelton, CT). Radioactivity profiles were prepared by plotting the resulting net CPM values vs HPLC time and radiochromatograms were reconstructed from the Topcount data using Microsoft® Excel software.

Metabolite identification. Metabolites in rat plasma, milk, urine, and fecal samples were analyzed as described previously (Zhang et al., 2009). LC/MS/MS analyses were performed on a LTQ mass spectrometer (ThermoFinnigan, San Jose, CA) with an ESI probe and an Agilent 1100 series HPLC system equipped with two pumps, an autoinjector, and a UV detector (Agilent, DE). The HPLC separation of the samples was performed using an ACE C18 column (3 μm, 4.6 x 150 mm). Samples were analyzed in the positive ionization mode and the capillary temperature was set at 280°C. The flow rate of nitrogen gas, spray current, and voltages were adjusted to give maximum sensitivity for the apixaban. The HPLC mobile phases and running conditions were the same as listed in the metabolite profile section.

Data analysis. Pharmacokinetic parameters in blood, plasma, and milk were calculated from the whole-body autoradiogram data or LSC data. These parameters included area under the concentration time curve from time 0 to the last measurable time point (AUC_{0-t}), and area under the concentration-time curve from 0 to infinity (AUC_{0-inf}), the maximal

concentration (C_{max}), T_{max} (the time to reach C_{max}), and half-life time ($t_{1/2}$). Pharmacokinetic parameters were calculated by using WinNonlin Professional Edition, version 4.1 (Pharsight, Mountain View, CA).

RESULTS

Tissue distribution of radioactivity. After single oral doses of [^{14}C]apixaban (5 mg/kg) to rats, the distribution of radioactivity in rat tissues was determined using QWBA and the results are listed in Tables 1-3. As shown in Figures 1A, B, and C, the distribution of [^{14}C]apixaban-derived radioactivity was extensive in the tissues of male, female, and pregnant rats. The general trend of radioactivity concentration determined in rat tissues was slightly higher in pregnant rats than that in male and female rats. In all rats, the highest radioactivity appeared in gastrointestinal tracts and followed by liver, kidney, adrenal gland, blood, adipose, lung, heart, salivary gland, pancreas, thymus, muscle, and other tissues (Tables 1-3 and Figures 1A, B, and C) and the lowest radioactivity appeared in brains. The C_{max} of radioactivity in most tissues was reached at 1 h postdose. After 8 h postdose, radioactivity was eliminated quickly in kidney, lung, heart, spleen, and other tissues. At 24 h after dosing, nearly the entire administered radioactivity was eliminated from the rat body. No significant differences in tissue retention of [^{14}C]apixaban were seen among the male, female, and pregnant rats and radioactivity in all rat tissues was below the limit of quantitation or undetectable at 48 h postdose.

After a single oral dose of [^{14}C]apixaban to pregnant rats, only low levels of radioactivity were detected in fetal tissues (Table 3 and Figure 1C). Fetal brains showed the lowest level of radioactivity. The C_{max} of radioactivity in fetal tissues was reached at 4 h postdose, then the radioactivity concentrations in tissues of fetuses declined quickly and were below the

limit of quantitation at 24 h postdose. At 48 h postdose, the overall radioactivity in maternal rats and fetuses was below the quantitation limit. The result also showed that radioactivity in amnion membrane was higher than other fetal tissues in all time points from 0.5 to 24 h (Figure 1C).

Pharmacokinetics of tissue distribution. After oral administration of [^{14}C]apixaban to rats, the pharmacokinetic parameters (T_{max}, $C_{max,}$ $t_{1/2,}$ and AUC) of radioactivity in blood were determined and the results are presented in Table 4. The results showed that the C_{max} of radioactivity in blood was reached at 0.5 to 1 h (T_{max}) postdose. After the peak concentrations were achieved in blood (C_{max} was 1180, 880, 1440, and 514 ng eq/g for male, female, pregnant and lactating rats, respectively), the blood radioactivity then declined in a log-linear manner until the end of the study at 168 h with a rapid elimination ($t_{1/2}$ ranged from 1.7 to 2.9 h). The results showed that pharmacokinetic parameters were similar between male and female rats. However, the AUC value was higher in pregnant rats (7200 ng eq•h/g) and lower in lacatating rats (1940 ng eq•h/g) than in male and female rats (4300 and 3780 ng eq•h/g, respectively).

Milk excretion. After a single oral dose of [^{14}C]apixaban at 5 mg/kg (150 μCi/kg) to lactating rats, [^{14}C]apixaban-derived radioactivity was extensively excreted in milk and was detected at all time points through 24 h postdose. The radioactivity concentration of apixaban-equivalent in milk at each time point was greater than that in blood or plasma. The T_{max} value of radioactivity in milk (6 h) was later than that in plasma and blood (Table 4). The elimination $t_{1/2}$ was similar between plasma and milk (Table 4). The milk/plasma concentration ratios ranged from 2.8 to 37. The milk $AUC_{0\text{-inf}}$ was approximately 30-fold greater than the plasma $AUC_{0\text{-inf}}$ (Table 4). The time-concentration profiles of radioactivity

in plasma, blood, and milk are shown in Figure 2A. Apixaban was the predominant component (>96%) in milk samples (Figure 2B). The average body weight of female rats used in this study was 270 g and the apixaban concentration in the collected milk was 2.75, 4.56, 7.59, 8.92, 3.61, and 0.31 μg/mL at 0.5, 1, 2, 6, 12, and 24 h, respectively. The time-averaged apixaban concentration in the 0-24 h milk was 4.6 μg/mL.

Routes of elimination. Following single oral dose of [^{14}C]apixaban, the average recovery of total radioactivity in rat urine and feces over the 0-168 h collection period was more than 89%. Fecal excretion accounted for about 74% (range from 68.6 to 77.6%) of radioactive dose. Approximately 14% of radioactive dose was recovered in urine.

Metabolic profile and identification. Metabolites were profiled in plasma, milk, urine, and feces, and the prominent peaks were investigated based on the radiochromatographic profiles with mass spectrometry. Mass spectra were compared to the synthesized standards of apixaban, *O*-demethyl apixaban, and previously identified metabolites (Zhang et. al., 2009).

The plasma profiling showed that the parent compound was the major component (representing 97% of plasma radioactivity) and metabolites were trace level compared with the parent drug in the 1 h plasma sample, and metabolic profiles of the plasma samples at 4, 12, and 24 h were qualitatively similar to 1 h plasma sample (data not shown).

Figure 2B shows the radioactivity profiles of milk samples at 1, 6, 12, and 24 h after single oral doses of [^{14}C]apixaban to rats. Apixaban was the major component, representing >96% of sample radioactivity. The profiles were qualitatively similar among milk samples at all time points and metabolites were minor compared with the parent drug.

The predominant radioactive peak in rat urine was apixaban (93%). The remaining radioactivity was distributed among three minor radioactive peaks, including M2 (*O*-demethyl apixaban, 2%), M4 (hydroxy apixaban, 2%), and M7 (3-hydroxy apixaban, 1.3%). The major radioactivity peak in feces was parent compound (83%). The minor metabolites in feces included M2 (13%), M4 (0.6%), and M7 (2.6%), similar to the metabolite profile reported previously (Zhang et al., 2009).

DISCUSSION

The purpose of this study was to investigate the tissue distribution and elimination of [^{14}C]apixaban in male, female, pregnant, and lactating rats following single oral doses. Following single oral administration, absorption of apixaban was rapid in rats and reaching C_{max} at 1 h in blood and most organs. The radioactivity in blood and most tissues declined quickly with no detectable radioactivity at 24 h postdose. The recovery of radioactivity averaged 74% in feces and only about 14% in urine, suggesting that fecal excretion was the major elimination route for apixaban in rats. Metabolite profiling showed that the parent drug was the major component in rat plasma, milk, urine, and feces with several minor metabolites including M2, M4, and M7 present in urine (accounted for about 5% of dose) and feces (accounted for about 17% dose). Based on these results and those previously reported (Zhang et al., 2009), tissue distribution and elimination of radiolabel largely reflects that of unchanged apixaban in rats.

The distribution of radioactivity of [^{14}C]apixaban in rats was extensive and the distribution patterns were similar among male, female, and pregnant rats with limited placenta-fetal transfer. These results are consistent with a high permeability of apixaban in a parallel artificial membrane permeability assay assay (data not shown), which would lead to

passive diffusion across lipid membranes. During all the experimental period, the highest radioactivity was observed in gastrointestinal (GI) tract and liver, which was consistent with the oral route administration and fecal excretion as a major elimination pathway of apixaban in rats. Radioactivity concentrations were also higher in rat kidney and urinary bladder, consistent with renal excretion as an important elimination pathway for apixaban in rats. Autoradiograms showed that radioactivity level was considerably lower in rat brain than for other well-perfused organs (e.g. heart, lung and muscle), suggesting that apixaban had a limited penetration through the blood-brain barrier. Several mechanisms could play a role for this observation, including efflux transporters in the blood-brain barrier. Several efflux transporters such as P-glycoprotein (P-gp) and the breast cancer resistance protein (BCRP) present in the blood-brain barrier and prevent or reduce drug entry (Bart et al., 2000; Bendayan et al., 2002; Cooray et al., 2002; Cisternino et al., 2004; Demeule et al., 2002; Golden and Pollack, 2003). The interactions of apixaban with efflux transporters in the blood-brain barrier could be a mechanism for the low level radioactivity in rat brain since apixaban was a substrate of P-gp and BCRP (unpublished results).

In pregnant rats, the peak level of radioactivity in fetal blood was approximately 35% of that in maternal blood and the overall exposure was lower in fetal organs than comparative organs in the mother (Table 3 and Figure 1C). Studies have showed that the placenta membrane offered a protective barrier for the developing fetus by reducing the entry of drugs from mother to fetus (Syme et al., 2004). Efflux transporters include BCRP, P-gp, and multidrug resistance protein 2 (Mrp 2) can transport xenobiotics/drugs from the fetal compartment to the maternal circulation and protect the fetus from potential toxicity (Lankas et al., 1998; Jonker et al., 2000; Ceckova-Novotna et al., 2006). BCRP is the most

abundant transporters expressed in the placenta (Maliepaard et al., 2001). In human placenta, the mRNA level of BCRP was found to be even 10 times greater than that of P-gp in placenta (Ceckova et al., 2006), and the mRNA expression in placenta was 100 times greater than in heart, lung, muscle, kidney, spleen, thymus and pancreas (Doyle et al., 1998) . Therefore, it is possible that BCRP plays an important role in the limited transfer of apixaban maternally administered drugs to the fetus.

Drug transfer into breast milk is determined by many factors such as ionization, plasma protein binding, molecular weight, lipophilicity of the drug, and its pharmacokinetics in the mother. In vivo animal studies showed that the excretion of some drugs into rat milk was due to the active transport mechanism (Alcorn and McNamara 2002; McNamara et al., 1992; McNamara et al. 1996; Gerk et al., 2001). Apixaban is a non-ionizable molecule. Our results showed that apixaban was extensively secreted into milk following a single oral dose of [^{14}C]apixaban to lactating rats. The average milk consumption is 5 mL/day for the first 5 days after birth (Romero et al., 1975). Ten baby rats would consume 50 ml of milk per day, which would represent approximately 10% dose (average milk concentration of apixaban times milk volumes/ average apixaban dose). Milk secretion has not been recognized as an elimination pathway of a drug, which is, however, reasonable during lactation period for any compound that shows significant milk secretion. The lowest blood concentrations and AUC values in the lactating rats compared to other groups of rats (Table 4) could be due to milk excretion as an additional clearance pathway. BCRP is strongly induced in the mammary gland of mice, cows, and humans during lactation and is responsible for the active secretion of clinically and toxicologically important substrates (Jonker et al., 2005). In comparison, other efflux transporters such as P-gp, Mrp1 and Mrp

2 were found to be absent from breast tissue in lactating mouse, suggesting these efflux transporters may not be as important as BCRP in terms of lacteal secretion. The list of BCRP substrates has grown quite large in recent years (Polgar et al., 2008), suggesting that drug secretion into breast milk may occur more commonly than previously thought.

In summary, after oral administration of [^{14}C]apixaban, the tissue distribution of apixaban was extensive in male, female, and pregnant rats, but with limited transfer to brain and fetal tissues. There was extensive secretion of apixaban into milk. The parent drug was the predominant component in rat plasma, milk, urine, and feces. Tissue distribution and drug excretion of apixaban are consistent with a moderately permeable drug that is a substrate for P-gp and BCRP efflux transporters.

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Authorship contribution:

Participated in design: Wang, He, Humphreys, Zhang

Conducted experiments: Wang, Zhang

Contributed new reagents and analytical tools: Maxwell

Data analysis: Wang, Tremaine, Grossman, Humphreys, Zhang

Contributed to writing: Wang, Tremaine, Grossman, Humphreys, Zhang

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Legends for figures:

Figure 1A. Representative whole-body autoradiogram of radioactivity distribution in a male rat at 1 h following single PO administration of [^{14}C]apixban (5 mg/kg).

Figure 1B. Representative whole-body autoradiogram of radioactivity distribution in a female rat at 1 h following single PO administration of [^{14}C]apixban (5 mg/kg).

Figure 1C. Representative whole-body autoradiogram of radioactivity distribution in a pregmamt rat at 1 h following single PO administration of [^{14}C]apixban (5 mg/kg).

Figure 2A. Radioactivity profiles of blood, plasma, and milk at specified times after single oral administration of [^{14}C]apixaban (5 mg/kg) to rats.

Figure 2B. Radioactivity profiles of rat milk samples at 1, 6, 12, and 24 h after single oral administration of [^{14}C]apixaban (5 mg/kg).

Table 1. Tissue distribution of radioactivity in male rats following a single oral dose of [^{14}C]apixaban at 5 mg/kg

Tissue	μg eq of apixaban/g					
	0.5 h	1 h	4 h	8 h	24 h	72 h
Adrenal gland	0.59	1.20	0.76	0.17	NS	NS
Blood	0.42	1.18	0.60	0.08	NS	NS
Bone marrow	0.26	0.57	0.32	0.07	NS	NS
Brain	0.05	BLQ	BLQ	BLQ	BLQ	BLQ
Brown fat	0.46	0.94	0.53	0.07	NS	NS
Harderian gland	0.21	0.69	0.42	0.12	ND	ND
Heart	0.33	0.70	0.45	0.08	ND	ND
Kidney (cortex)	0.81	1.72	1.36	0.88	BLQ	ND
Liver	0.81	1.72	1.36	0.39	BLQ	ND
Lung	0.57	0.70	0.64	0.14	BLQ	ND
Muscle	0.20	0.43	0.27	0.06	BLQ	ND
Pancreas	0.47	0.62	0.55	0.12	BLQ	ND
Pituitary gland	0.26	0.59	0.37	0.07	ND	ND
Salivary gland	0.30	0.72	0.54	0.13	BLQ	ND
Spleen	0.18	0.32	0.28	0.09	ND	ND
Thymus	0.16	0.46	0.23	0.06	ND	ND

ND: not dectectable; BLQ, below limit of quantitation (0.037 μg eq/g tissue).

NS: not sampled, since not visualized on autoradiograph, considered as BLQ.

Table 2. Tissue distribution of radioactivity in female rats following a single oral dose of [^{14}C]apixaban at 5 mg/kg

Tissue	μg eq of apixaban/g					
	0.5 h	1 h	4 h	8 h	24 h	72 h
Adrenal gland	0.56	1.10	0.90	0.30	ND	ND
Blood	0.40	0.88	0.35	0.17	ND	ND
Bone marrow	0.29	0.41	0.26	0.15	ND	ND
Brain	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Brown fat	0.48	0.77	0.61	0.17	ND	ND
Harderian gland	0.40	0.64	1.12	0.22	ND	ND
Heart	0.37	0.54	0.51	0.11	ND	ND
Kidney (cortex)	0.82	1.36	1.21	0.42	ND	ND
Liver	267	4.31	3.98	1.12	BLQ	ND
Lung	0.43	0.883	0.51	0.20	NS	NS
Muscle	0.23	0.27	0.29	0.08	ND	ND
Pancreas	0.43	0.62	0.55	0.19	ND	NS
Pituitary	0.43	0.42	0.48	0.22	ND	ND
Salivary gland	0.28	0.55	0.44	0.13	ND	ND
Spleen	0.29	0.35	0.45	0.13	ND	ND
Thymus	0.20	0.27	0.30	0.09	ND	ND

ND: not dectectable; BLQ, below limit of quantitation (0.037 μg eq/g tissue).

NS: not sampled, since not visualized on autoradiograph, considered as BLQ.

Table 3. Tissue distribution of radioactivity in pregnant rats following a single oral dose of [^{14}C]apixaban at 5 mg/kg

Tissue	μg eq of apixaban/g					
	0.5 h	1 h	4 h	8 h	24 h	48
Adrenal gland	1.49	1.90	2.30	0.18	NS	NS
Blood	1.43	1.44	1.30	0.09	ND	ND
Bone marrow	0.50	0.33	0.46	0.06	ND	ND
Brain	0.05	0.06	BLQ	BLQ	ND	ND
Brown fat	1.08	0.95	0.87	0.06	ND	ND
Harderian gland	1.00	1.09	1.1	0.19	ND	ND
Heart	0.68	0.93	0.83	0.06	ND	ND
Kidney (cortex)	2.11	2.03	1.93	0.21	0.05	ND
Liver	6.77	7.38	6.13	0.65	0.12	ND
Lung	1.65	1.56	1.33	0.15	BLQ	ND
Muscle	0.37	0.46	0.50	0.05	ND	ND
Pancreas	0.89	0.75	0.99	0.09	ND	ND
Pituitary	0.72	0.93	0.75	0.19	NS	NS
Salivary gland	0.73	0.95	1.03	0.08	ND	ND
Spleen	0.46	0.51	0.47	0.05	BLQ	ND
Thymus	0.38	0.58	0.54	0.05	ND	ND
Amnion	1.45	2.12	11.24	2.12	3.52	NS
Placenta	1.02	0.74	1.06	0.10	BLQ	ND
Fetal blood	0.27	0.31	0.51	0.07	ND	ND
Fetal brain	BLQ	0.05	0.06	BLQ	ND	ND
Fetal kidney	0.24	0.20	0.46	NS	NS	NS
Fetal liver	0.16	0.22	0.32	0.06	NS	NS

ND: not dectectable; BLQ, below limit of quantitation (0.037μg eq/g tissue).

NS: not sampled, since not visualized on autoradiograph, considered as BLQ.

Table 4: Pharmacokinetic parameters of radioactivity in blood and milk after a single oral dose of [^{14}C]apixaban (5 mg/kg) to rats

Rats	Sample	T_{max} (h)	C_{max} (ng eq/g)	$t_{½}$ (h)	$AUC_{(0-t)}$ (ng eq·h/g)	$AUC_{(0-\infty)}$ (ng eq·h/g)
Male	Blood	1.0	1180	1.7	4080	4300
Female	Blood	1.0	880	2.9	3110	3780
Pregnant	Blood	1.0	1440	1.7	6980	7200
Lactating	Blood	0.5	514	4.2	1940	1960
	Plasma	0.5	1040	4.3	3420	3470
	Milk	6.0	8920	3.7	103000	104000

Fig 1A

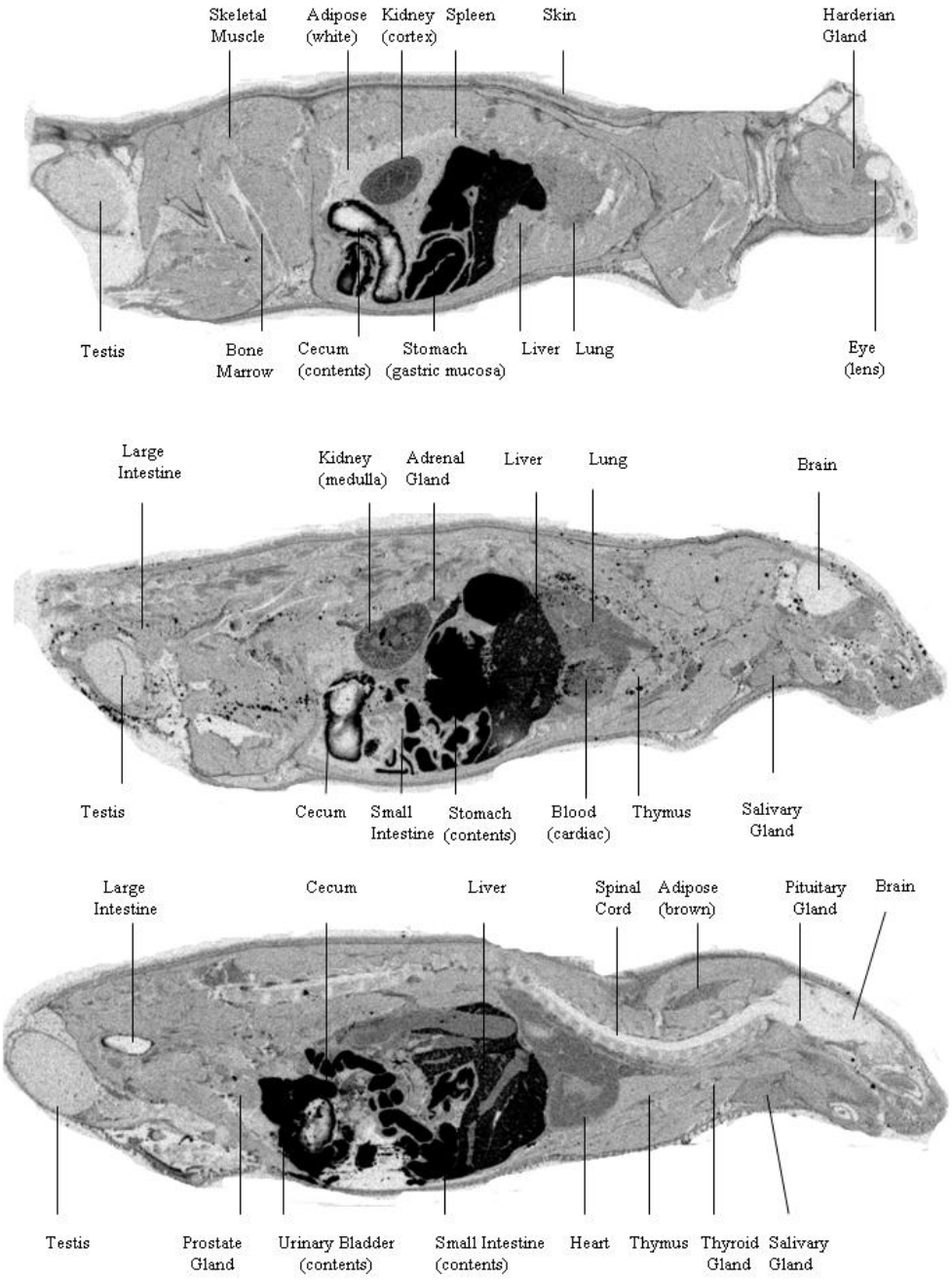

Skeletal Muscle
Adipose (white)
Kidney (cortex)
Spleen
Skin
Harderian Gland
Testis
Bone Marrow
Cecum (contents)
Stomach (gastric mucosa)
Liver
Lung
Eye (lens)
Large Intestine
Kidney (medulla)
Adrenal Gland
Liver
Lung
Brain
Testis
Cecum
Small Intestine
Stomach (contents)
Blood (cardiac)
Thymus
Salivary Gland
Large Intestine
Cecum
Liver
Spinal Cord
Adipose (brown)
Pituitary Gland
Brain
Testis
Prostate Gland
Urinary Bladder (contents)
Small Intestine (contents)
Heart
Thymus
Thyroid Gland
Salivary Gland

Fig 1B

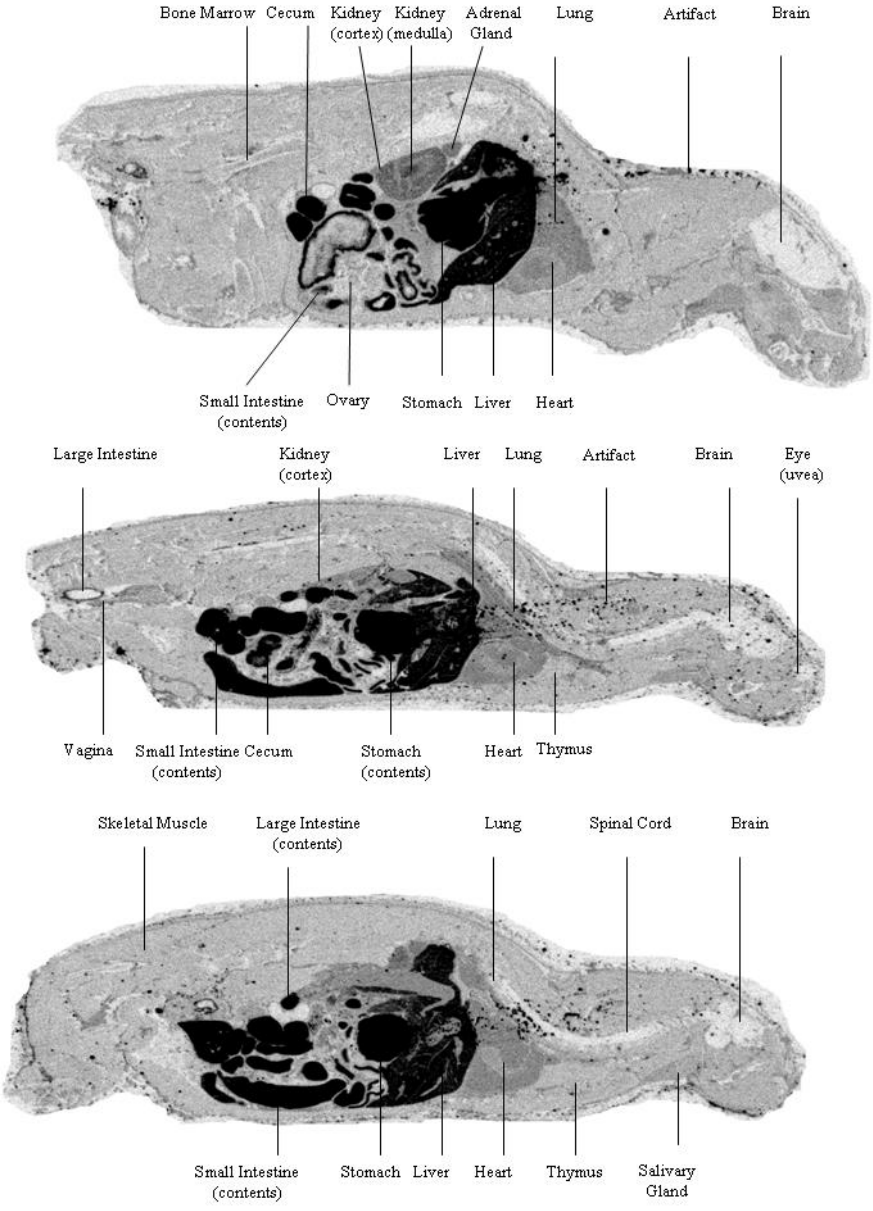

Bone Marrow
Cecum
Kidney (cortex)
Kidney (medulla)
Adrenal Gland
Lung
Artifact
Brain
Small Intestine (contents)
Ovary
Stomach
Liver
Heart
Large Intestine
Kidney (cortex)
Liver
Lung
Artifact
Brain
Eye (uvea)
Vagina
Small Intestine (contents)
Cecum
Stomach (contents)
Heart
Thymus
Skeletal Muscle
Large Intestine (contents)
Lung
Spinal Cord
Brain
Small Intestine (contents)
Stomach
Liver
Heart
Thymus
Salivary Gland

Fig 1C

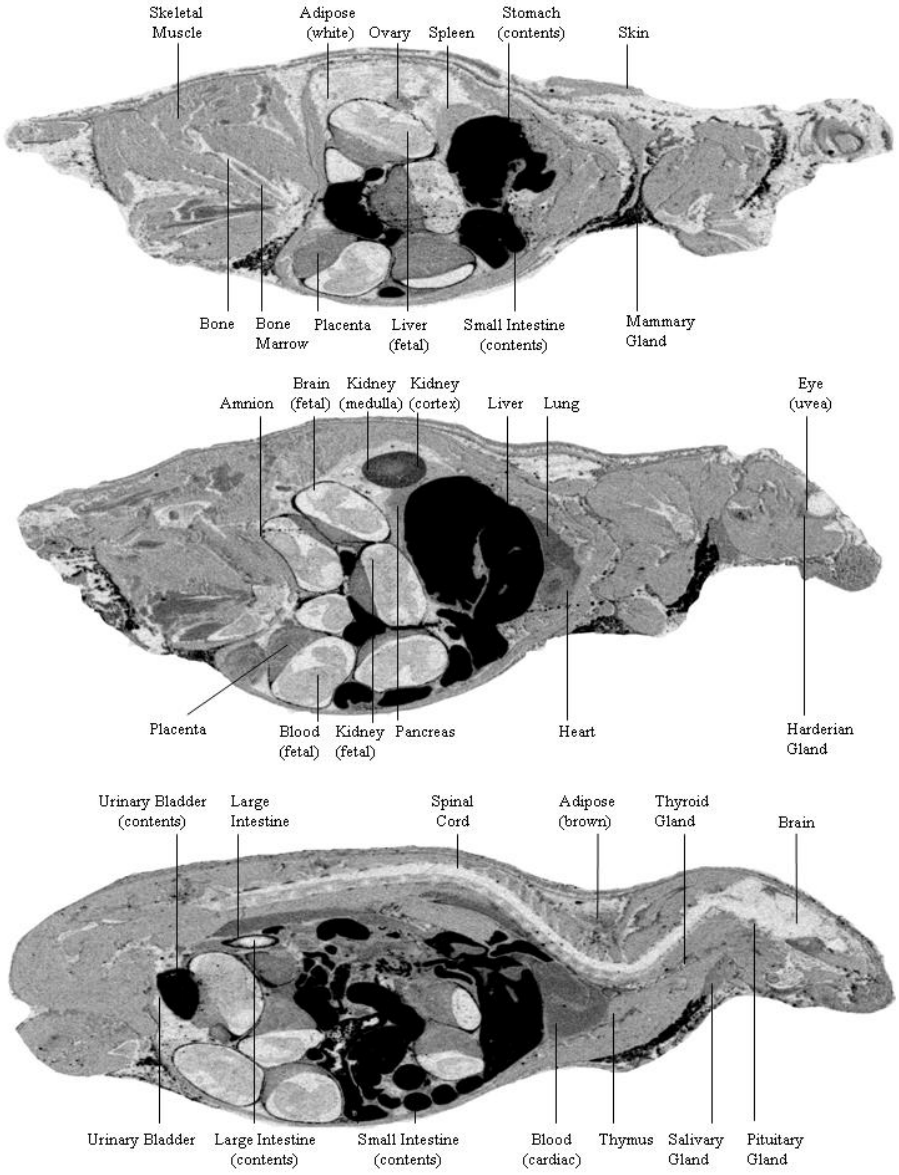

Skeletal Muscle
Adipose (white)
Ovary
Spleen
Stomach (contents)
Skin
Bone
Bone Marrow
Placenta
Liver (fetal)
Small Intestine (contents)
Mammary Gland
Amnion
Brain (fetal)
Kidney (medulla)
Kidney (cortex)
Liver
Lung
Eye (uvea)
Placenta
Blood (fetal)
Kidney (fetal)
Pancreas
Heart
Harderian Gland
Urinary Bladder (contents)
Large Intestine
Spinal Cord
Adipose (brown)
Thyroid Gland
Brain
Urinary Bladder
Large Intestine (contents)
Small Intestine (contents)
Blood (cardiac)
Thymus
Salivary Gland
Pituitary Gland

Fig 2A

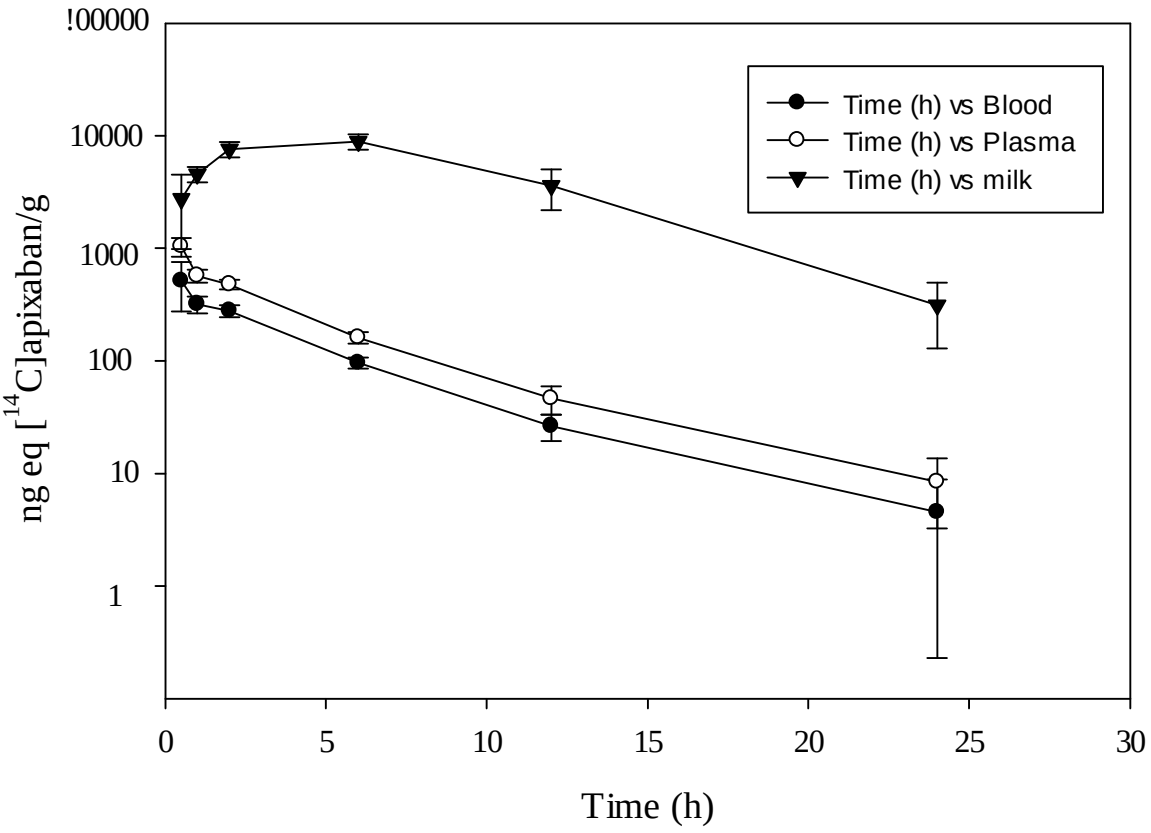
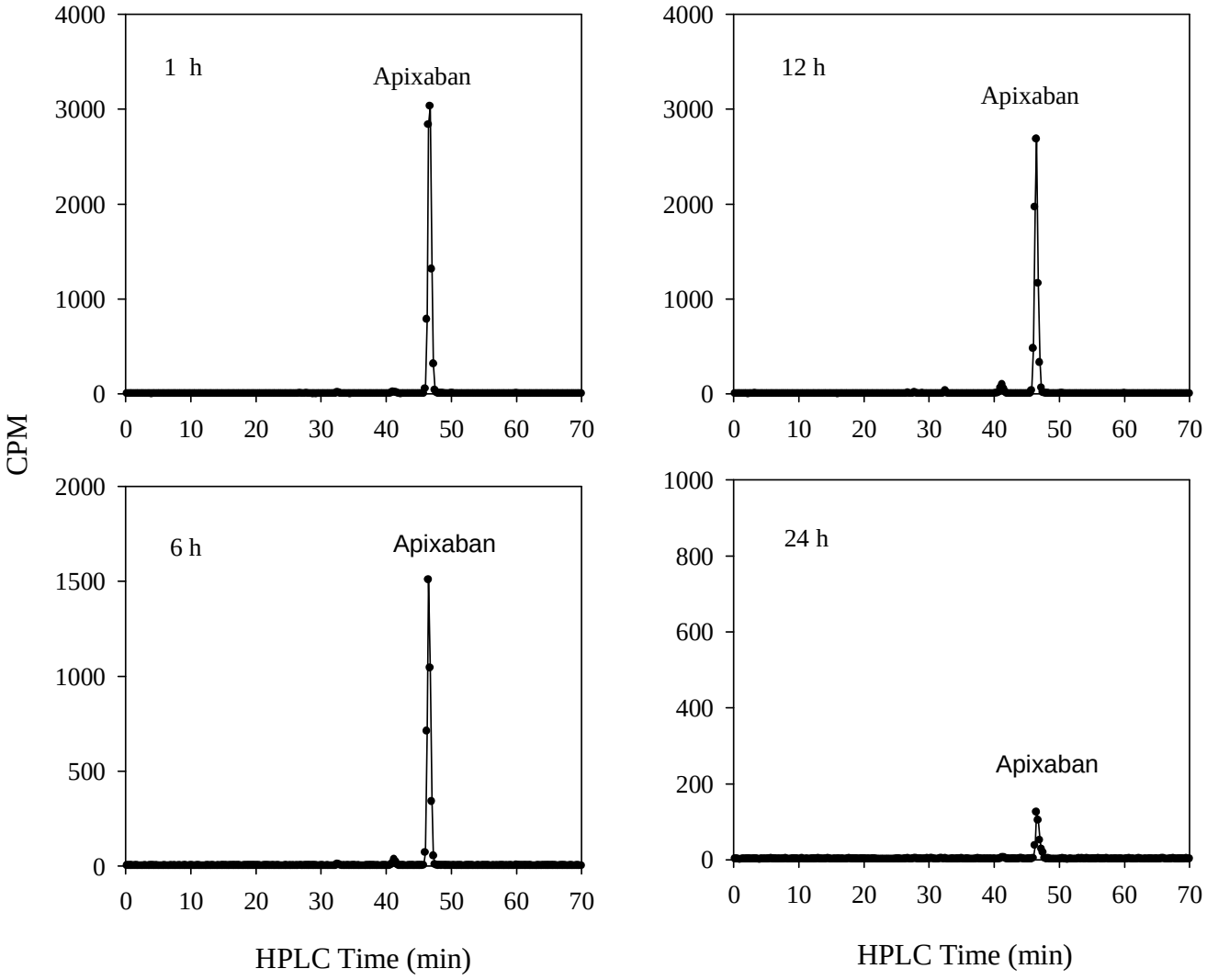

!00000
10000
1000
100
10
1
ng eq [14C]apixaban/g
0
5
10
15
20
25
30
Time (h)
Time (h) vs Blood
Time (h) vs Plasma
Time (h) vs milk

Fig 2B


1 h
Apixaban
12 h
Apixaban
6 h
Apixaban
24 h
Apixaban
CPM
HPLC Time (min)
HPLC Time (min)