Disposition and Metabolism of Ticagrelor, a Novel P2Y₁₂ Receptor Antagonist, in Mice, Rats, and Marmosets

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ABSTRACT:

Ticagrelor is a reversibly binding and selective oral P2Y₁₂ antagonist, developed for the prevention of atherothrombotic events in patients with acute coronary syndromes. The disposition and metabolism of [¹⁴C]ticagrelor was investigated in mice, rats, and marmosets to demonstrate that these preclinical toxicity species showed similar metabolic profiles to human. Incubations with hepatocytes or microsomes from multiple species were also studied to compare with in vivo metabolic profiles. The routes of excretion were similar for both oral and intravenous administration in mice, rats, and marmosets with fecal excretion being the major elimination pathway accounting for 59 to 96% of the total radioactivity administered. Urinary excretion of drug-related material accounted for only 1 to 15% of the total radioactivity administered. Milk samples from lactating rats displayed significantly higher levels of total radioactivity than plasma after oral administration of ticagrelor. This demonstrated that ticagrelor and/or its metabolites were readily transferred into rat milk and that neonatal rats could be exposed to ticagrelor-related compounds via maternal milk. Ticagrelor and active metabolite AR-C124910 (loss of hydroxethyl side chain) were the major components in plasma from all species studied and similar to human plasma profiles. The primary metabolite of ticagrelor excreted in urine across all species was an inactive metabolite, AR-C133913 (loss of difluorophenylcyclopropyl group). Ticagrelor, AR-C124910, and AR-C133913 were the major components found in feces from the three species examined. Overall, in vivo metabolite profiles were qualitatively similar across all species and consistent with in vitro results.

Introduction

Platelet activation and aggregation play important roles in occlusive vascular events (Davies et al., 1986; Fitzgerald et al., 1986). Release of ADP from activated platelets is one of the primary mediators of platelet aggregation, leading to a sustained response via activation of P2Y₁₂ receptors (Schrör, 1995; Gershlick, 2000). Inhibition of platelet aggregation with a combination of aspirin and a thienopyridine antiplatelet drug, such as clopidogrel, is an important strategy for preventing ischemic events in patients with acute coronary syndromes (Yusuf et al., 2001; Bassand et al., 2007). However, there is still a need for treatment regimens with improved efficacy.

Ticagrelor [(1S,2S,3R,5S)-3-[7-[[1R,2S]-2-(3,4-difluorophenyl)cyclopropyl]amino]-5-propylthio]-3H-1,2,3-triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)-1,2-cyclopentanediol (AZD6140)] (Fig. 1) is a member of the new class of antiplatelet agents known as cyclopentyl-triazolo-pyrimidines, which require activation via cytochrome P450 oxidation to become ring-open thiol containing pharmacologically active metabolites such as clopidogrel in important ways. Ticagrelor is a reversibly binding P2Y₁₂ antagonist, whereas the thienopyridines (clopidogrel, prasugrel, ticlopidine) bind irreversibly to this receptor. Unlike thienopyridines, which require activation via cytochrome P450 oxidation to become ring-open thiol containing pharmacologically active metabolites (Farid et al., 2010), ticagrelor acts directly on the P2Y₁₂ receptor without requiring metabolic activation.

Ticagrelor selectively inhibits the platelet P2Y₁₂ receptor to block ADP’s prothrombotic effects. It has been demonstrated that ticagrelor nearly completely inhibited ADP-induced platelet aggregation ex vivo (Husted et al., 2006), and 100 mg of ticagrelor b.i.d. in humans demonstrated 97, 93, and 93% inhibition of platelet aggregation at 4, 12, and 24 h, respectively (Tantry et al., 2007). Ticagrelor is mechanistically differentiated from thienopyridine antiplatelet agents such as clopidogrel in important ways. Ticagrelor is a reversibly binding P2Y₁₂ antagonist, whereas the thienopyridines (clopidogrel, prasugrel, ticlopidine) bind irreversibly to this receptor. Unlike thienopyridines, which require activation via cytochrome P450 oxidation to become ring-open thiol containing pharmacologically active metabolites (Farid et al., 2010), ticagrelor acts directly on the P2Y₁₂ receptor without requiring metabolic activation.

Ticagrelor does not require metabolic activation for its antiplatelet activity, but it does have an active metabolite, AR-C124910 (loss of hydroxethyl side chain) (Fig. 1). This metabolite has approximately equal potency at the P2Y₁₂ receptor as ticagrelor and is present in the circulation at approximately one third of the concentration of the parent drug (Husted et al., 2006). In healthy human subjects and patients with stable atherosclerosis, the pharmacokinetics of ticagrelor are linear and predictable over a wide dose range (Husted et al., 2006; Teng and Butler, 2008; Butler and Teng, 2010). Ticagrelor and...
Materials and Methods

Drugs, Standards, and Reagents. Ticagrelor (AZD6140) and synthetic standards of metabolites AR-C124910 and AR-C133913 were synthesized by Medicinal Chemistry at AstraZeneca (Wilmington, DE or Charnwood, Loughborough, UK). The synthesis of ticagrelor (AZD6140) has been reported previously (Sprinthorpe et al., 2007). [14C]Ticagrelor (55 mCi/mmol, 97% radiochemical purity) was synthesized by GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). All of the chemicals were obtained from Sigma-Aldrich (St. Louis, MO) unless specified. Solvents used for high-performance liquid chromatography (HPLC) and mass spectrometry (MS) analysis were of HPLC grade. Hepatocytes were freshly prepared from a male Sprague-Dawley rat and a male Beagle dog after a standard collagenase-based digestion procedure (Seglen, 1976). Two cryopreserved preparations of human hepatocytes (lots HRK and 117) were obtained from In Vitro Technologies (Baltimore, MD). Microsomal fractions were prepared from fresh liver tissues using differential centrifugation of tissue homogenates (Amar-Costescu et al., 1974). Microsomes were stored frozen at approximately −70°C until use. Mouse (CD-1, male), rat (Sprague-Dawley, male), marmoset monkey (male), beagle dog (male), and pooled human liver microsomes were all prepared in house. Cynomolgus monkey (male) liver microsomes (lot 0210400) were purchased from XenoTech, LLC (Lenexa, KS).

Excretion Mass Balance of [14C]Ticagrelor in Animals. Excretion mass balance studies of [14C]ticagrelor were conducted in rat, mice, and marmosets after single oral and intravenous doses at Charles River Laboratories (Tranent, UK). Plasma was separated from blood collected by centrifugation within 20 min of sampling. The [14C]ticagrelor oral dose formulations were prepared in 1% carboxymethylcellulose/0.1% Tween 80 in water. The intravenous dosing solution was prepared in 2% Tween 80 for all species.

Mice. Twelve male and 12 female CD-1 mice (Charles River Laboratories) were divided into four groups (3 male and 3 female mice in each group). The oral and intravenous dosing solutions were 2.08 and 1.20 mg/ml, respectively. Two groups were given an oral gavage dose (2.08 and 1.20 mg/kg, 459 µCi/kg) of [14C]ticagrelor, and two groups were given a dose via a tail vein injection (6 mg/kg, 459 µCi/kg) of [14C]ticagrelor. For each dose route, one group was used for the separate collection of urine, feces, and cage wash. Urine was collected before dose and at 24-h intervals to 72 h after dose. Feces were collected at 24-h intervals to 72 h after dose. The cages were washed at the time of each feces collection, and the wash was retained. The final cage wash (72 h) was carried out using ethanol/water (50:50, v/v). Terminal blood samples (2 h after dose) were collected from the remaining group of three male and three female mice that each received either a single oral or a single intravenous administration of [14C]ticagrelor at a target dose level of 20 or 6 mg/kg, respectively.

Rats. Two groups of Sprague-Dawley rats (Charles River Laboratories) (three males and three females in each group) were administered [14C]ticagrelor orally by gavage at a target dose of 20 mg/kg (49.9 µCi/kg) or intravenously via tail vein injection at a target dose level of 3 mg/kg (49.9 µCi/kg). The oral and intravenous dosing solutions were 4.07 and 1.18 mg/ml, respectively. For each dosing group, urine was collected for the periods of 0 to 6, 6 to 12, and 12 to 24 h and then at 24-h intervals until 120 h after dose. Feces were collected at 24-h intervals to 120 h after dose. The cages were washed at the time of each feces collection, and the wash was retained. To collect blood samples for pharmacokinetic studies, 11 groups of three male and three female rats each received a single oral administration of [14C]ticagrelor at a target dose level of 20 mg/kg, and 12 groups of three male and three female rats each received a single intravenous administration of [14C]ticagrelor at a target dose level of 3 mg/kg. Three male and three female animals were sacrificed by CO2 narcosis at each of the following time points after dose: 0.25 (intraocular only), 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h.

The dosing and radioactivity measurement in rat milk was carried out in Charles River Laboratories. Eight time-mated female Sprague-Dawley rats, approximately 9 weeks of age at mating (body weight, 267–342 g at dosing), were given a single oral administration of [14C]ticagrelor at a target dose of 60

human liver microsomes to establish the complete metabolism picture of ticagrelor in vitro and to provide comparison to metabolite profiles observed in vivo.

FIG. 1. Chemical structures of ticagrelor and its major metabolites AR-C124910 (M8) and AR-C133913 (M5).
mg/kg on day 11 after parturition and were placed singly in polypropylene and stainless steel cages with nesting material. Groups of two rats were used to collect milk and maternal plasma at each time point of 1, 4, 24, and 48 h after dose.

**Marmosets.** Ten male and 10 female healthy, laboratory-bred marmoset monkeys were obtained from Harlan UK Limited (Bicester, Oxon, UK). The oral and intravenous dosing solutions were 4.39 and 1.66 mg/ml, respectively. Each marmoset monkey was given a targeted oral dose (20 mg/kg, 105.7 µCi/kg) of [14C]ticagrelor via gastric gavage and split into two groups. After a period of 21 days, the same 20 animals were given an intravenous dose administration via the saphenous vein at a targeted dose of 3 mg/kg (83.3 µCi/kg) and divided into groups as described previously. After an additional wash-out period of 42 days, two male animals were given an oral administration (20 mg/kg, 105.7 µCi/kg), and urine, feces, and expired air were collected. At the end of the collection period, these two animals were sacrificed, and the carcasses were retained. Urine was collected (into containers cooled by solid CO2 for the first 48 h) for the periods of 0 to 6, 6 to 12, and 12 to 24 h and then at 24-h intervals until 168 h after dose. Feces were collected at 24-h intervals to 168 h after dose. The cages were washed at the time of each urine collection, and the wash was retained. Cage debris (wasted food) was also retained. Whole blood samples (−0.5 ml) were collected from the cephalic or femoral veins, alternately from two groups of three male and three female marmosets, into heparinized tubes at the following time points for both the oral and intravenous dosing groups: 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after dose.

**In Vitro Species Metabolite Profiles Comparison in Hepatocytes and Microsomes.** Hepatocytes. [14C]Ticagrelor (20 µM) was incubated with freshly isolated hepatocytes from rat, dog, and cryopreserved hepatocytes from human (2 × 10^9 cells/ml) for 4 h at pH 7.4 at 37°C with gentle shaking in a 10 cm² flask that was maintained under an oxygen/carbon dioxide (95:5) and 95% humidity atmosphere. Hepatocyte incubation mixtures contained Williams E medium (with 25 mM HepES), 1% insulin-transferrin-selenium solution (catalog number 41400-045; Invitrogen, Carlsbad, CA), 10 mM HepES, and 2 mM L-glutamine. [14C]-7-Ethoxycoumarin was used as quality control for phase I and II metabolic reactions and was incubated for 4 h at a concentration of 25 µM.

Microsomes. Incubations of [14C]ticagrelor were performed with liver microsomes from mouse, rat, dog, marmoset, cynomolgus monkey, and human. [14C]Ticagrelor (20 µM) was incubated with 0.5 mg/ml protein, 100 mM potassium phosphate buffer (pH 7.4), 5 mM MgCl2, and a NADPH-regenerating system (5 mM glucose-6-phosphate, 5 mM NADP⁺, and 1 U/ml glucose-6-phosphate dehydrogenase). Metabolic reactions were terminated at 60 min by the addition of an equal volume of acetonitrile with 0.1% formic acid to each incubation.

After termination of metabolic reactions, all samples were centrifuged, and the supernatant was transferred to HPLC vials for LC-MS/MS analysis or amber glass vials for storage. The pellet was washed with 2 ml of acetonitrile (with 0.1% formic acid) twice. Triplicate aliquots were taken from the original supernatant and the two wash solutions for liquid scintillation counting (LSC). Samples were analyzed by LC-MS/MS as described under Sample Analysis.

**Determination of Total Radioactivity.** Radioactivity was determined using a Packard TR 2100 Liquid Scintillation Analyzer (Packard Biosciences Limited, Waltham, MA) with automatic quench correction by an external standardization method. Duplicate aliquots of plasma samples were made up to 1 ml with water (if necessary) and mixed with Quicksint 1 scintillation fluid (10 ml; Zinsser Analytic, Mainz, Germany). Duplicate aliquots of urine samples were made up to 1 ml with water (if necessary) and mixed with Quicksint 1 scintillation fluid (10 ml). Feces samples were homogenized in ethanol, where appropriate, and duplicate portions of each sample (−0.3 g) combusted using a Packard Tri-Carb 307 Automatic Sample Oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA). The resultant 14CO2 generated was collected by absorption in Carbosorb (8 ml), to which Permafluor E+ scintillation fluid (10 ml; PerkinElmer Life and Analytical Sciences) was added.

**Sample Preparation for Metabolite Characterization.** Plasma. Mouse plasma samples were prepared by combining a fixed volume (110 µl) of plasma from each subject (2 h after dose) for each gender and administration route. Rat plasma samples were pooled by equal volumes (1 ml) across time points for each gender and administration route (0.25–12 h for intravenous dose and 0.5–12 h for oral dose). Marmoset plasma samples were pooled across animals and time points (0.5–8 h) for each gender and administration route. Each pooled plasma sample was extracted using 2 to 3 volumes of acetonitrile/methanol (1:1, v/v). Precipitated proteins were removed by centrifugation. The radioactivity in each supernatant was determined by LSC. The supernatants were evaporated to dryness under a stream of nitrogen, and the residues were reconstituted in water or 50% acetonitrile/water. The samples were analyzed by LC-MS/MS as described under Sample Analysis.

Milk: Duplicate aliquots of rat milk and (plasma from this study) were made up to 1 ml with distilled water (if necessary) and mixed with Aquasafe 500 Plus scintillation fluid (10 ml; Zinsser Analytic) for radioactivity measurement by LSC. To 150 µl of each milk sample, 300 µl of acetonitrile was added. The samples were vortexed for 10 s, followed by centrifugation for 15 min at 20,000g, at +5°C. The supernatants were decanted, and 20 µl was analyzed by LC-MS/MS as described under Sample Analysis.

**Table 1. Mass Balance Results in Mice, Rats, and Marmosets after Single Oral or Intravenous Administration of [14C]Ticagrelor**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling Time (h)</th>
<th>Matric</th>
<th>Oral %</th>
<th>Intra%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice (3/group)</td>
<td>0–72</td>
<td>Urine</td>
<td>3.8</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>95.8</td>
<td>95.3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>101.7</td>
<td>98.7</td>
</tr>
<tr>
<td>Rats (3/group)</td>
<td>0–120</td>
<td>Urine</td>
<td>4.71</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>87.0</td>
<td>89.8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>93.3</td>
<td>95.9</td>
</tr>
<tr>
<td>Marmosets (2/group)</td>
<td>0–168</td>
<td>Urine</td>
<td>8.49</td>
<td>8.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>58.8</td>
<td>66.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>67.3</td>
<td>74.6</td>
</tr>
<tr>
<td>Human(n=6)</td>
<td>0–168</td>
<td>Urine</td>
<td>26.5 ± 4.1</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feces</td>
<td>57.8 ± 4.4</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>84.3 ± 5.5</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not available.

a Oral dose was targeted at 20 mg/kg for mice, rats, and marmosets and at 200 mg for human.

b Intravenous dose was targeted at 3 mg/kg for rats and marmosets and at 6 mg/kg for mice.

c Total includes carcass, gastrointestinal tract, cage wash, and expired air (1 animal per sex).

d Total includes cage wash and cage debris.

f Human data have been presented previously (Teng et al., 2010) and were included here for comparison only.
**TABLE 2**

**Proposed structures of [14C]ticagrelor metabolites and occurrence**

<table>
<thead>
<tr>
<th>Drug or Metabolites</th>
<th>m/z (M+H)&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Major Fragment Ions</th>
<th>Proposed Structures</th>
<th>Source</th>
</tr>
</thead>
</table>
| Ticagrelor           | 523                   | 495, 477, 453, 435, 415, 335, 293 | ![Proposed Structures](image1) | Mouse: plasma, feces, MLM  
Rat: plasma, milk, feces, RH, RLM  
Marmoset: plasma, feces, MMMLM  
Dog: DH, DLM  
Cyano monkey: CMLM  
Human*: HH, HLM, plasma, feces |
| M1                  | 387                   | 345, 327, 317, 255, 237, 185 | ![Proposed Structures](image2) | Mouse: urine  
Rat: urine  
Marmoset: urine  
Cyano monkey: CMLM  
Human*: plasma, urine |
| M2                  | 387                   | 369, 351, 341, 236, 222 | ![Proposed Structures](image3) | Mouse: urine  
Rat: urine  
Marmoset: urine  
Cyano monkey: CMLM  
Human*: plasma, urine |
| M3a                 | 503                   | 327                 | ![Proposed Structures](image4) | Marmoset: urine  
Human*: urine<sup>a</sup> |
| M4a                 | 547                   | 371                 | ![Proposed Structures](image5) | Marmoset: plasma, urine  
Human*: urine<sup>b</sup> |
| M5 (AR-C133913)     | 371                   | 343, 325, 301, 183  | ![Proposed Structures](image6) | Mouse: plasma, urine, feces, MLM  
Rat: plasma, milk, urine, feces, RH, RLM  
Marmoset: plasma, urine, feces, MMMLM  
Dog: DH, DLM  
Cyano monkey: CMLM  
Human*: HH, HLM, plasma, urine, feces |
| M6b                 | 655                   | 479                 | ![Proposed Structures](image7) | Dog: DH  
Human*: HH, urine<sup>b</sup> |
| M7a                 | 495                   | 477, 449, 432, 417  | ![Proposed Structures](image8) | Mouse: plasma, urine, feces, MLM  
Rat: milk, urine, feces, RH  
Marmoset: urine, feces  
Cyano monkey: CMLM  
Human*: HH, feces |
| M7b                 | 495                   | 467, 449, 431, 409, 391, 373 | ![Proposed Structures](image9) | Mouse: MLM  
Rat: urine, feces, RH  
Marmoset: urine, feces  
Dog: DH  
Cyano monkey: CMLM  
Human: HH |
| M7c                 | 495                   | 453, 435, 425, 357, 337  | ![Proposed Structures](image10) | Mouse: MLM  
Rat: feces  
Marmoset: feces  
Cyano monkey: CMLM |
<table>
<thead>
<tr>
<th>Drug or Metabolites</th>
<th>m/z (M+H)</th>
<th>Major Fragment Ions</th>
<th>Proposed Structures</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>M7d 495</td>
<td></td>
<td></td>
<td></td>
<td>Rat: feces Marmoset: urine, feces</td>
</tr>
<tr>
<td>M7e 495 467, 327, 257</td>
<td></td>
<td></td>
<td></td>
<td>Rat: feces Marmoset: feces</td>
</tr>
<tr>
<td>M9a 699 523</td>
<td></td>
<td></td>
<td></td>
<td>Rat: feces, RH Marmoset: plasma, feces Dog: DH Human: HH Mouse: feces</td>
</tr>
<tr>
<td>M10a 539 511, 493, 453, 435, 351</td>
<td></td>
<td></td>
<td></td>
<td>Rat: milk, plasma, urine, feces Marmoset: urine, feces</td>
</tr>
<tr>
<td>M10b 539 521, 493, 431, 413</td>
<td></td>
<td></td>
<td></td>
<td>Mouse: feces, MLM Rat: milk, urine, feces, RH Marmoset: urine, feces, MMLM Dog: DH, DLM Cyno monkey: CMLM Human*: HH, HLM, urine*</td>
</tr>
<tr>
<td>M10c 539 511, 469, 451, 431, 371, 343</td>
<td></td>
<td></td>
<td></td>
<td>Rat: feces Marmoset: feces</td>
</tr>
<tr>
<td>M10d 539 511, 493, 451, 431, 371, 343</td>
<td></td>
<td></td>
<td></td>
<td>Rat: feces Marmoset: feces</td>
</tr>
<tr>
<td>M11 343 301, 283, 237, 185</td>
<td></td>
<td></td>
<td></td>
<td>Mouse: urine Rat: urine Marmoset: urine</td>
</tr>
</tbody>
</table>
Urine. Urine samples were proportionally pooled by weight (5–10%) from 0 to 48 h for mouse and rat and from 0 to 120 h for marmoset of each gender and administration route group. Pooled urine samples were centrifuged. Supernatants were removed, and radioactivity in the supernatants was determined by LSC. Urine samples were analyzed by LC-MS/MS as described under Sample Analysis.

Feces. Feces samples were proportionally pooled by weight (5–20%) from 0 to 24 h for mouse and rat and from 0 to 120 h for marmoset of each gender and administration route group. The pooled feces samples were extracted three times with acetonitrile/methanol (1:1, v/v) using approximately 3 ml of solvent per gram of pooled feces sample. During each extraction, the sample was well mixed and centrifuged, and the supernatant was decanted. The supernatant was combined and partitioned three times against hexane saturated in methanol. Each extract was concentrated to dryness under a steady stream of nitrogen. The dried extract was then redissolved in acetonitrile/water (1:1, v/v) with the aid of brief sonication. Aliquots of the reconstituted extract were taken for LSC to determine procedural recovery of total radioactivity. Feces samples were analyzed by LC-MS/MS as described under Sample Analysis.

Sample Analysis. LC-MS/MS systems. Three LC-MS/MS systems were used for in vivo and in vitro metabolite characterization work. All in vitro and in vivo rat and marmoset samples except rat milk were analyzed by LC-MS/MS system 1; mouse samples were analyzed by LC-MS/MS system 2; rat milk samples were analyzed by LC-MS/MS system 3. LC-MS/MS system 1 consisted of a LCQ mass spectrometer (Thermo Fisher Scientific, Waltham, MA) with an electrospray ionization probe and a HP1100 LC system (Hewlett Packard, Palo Alto, CA) equipped with a pump, an autosampler, and a UV detector. Samples were analyzed in the positive ionization mode, and the capillary temperature was set at 180°C. The flow rate of nitrogen gas, spray detector. Samples were analyzed in the positive ionization mode, and the capillary temperature, 325°C; nitrogen sheath gas pressure, 50 psi; auxiliary gas pressure, 1.5 psi; source temperature, 250°C. The spray voltage was 5.0 kV; heated capillary temperature, 325°C; nitrogen sheath gas pressure, 50 psi; auxiliary gas pressure, 15 psi. The ion source sensitivity was optimized with ticagrelor, and positive ion mass spectra were acquired full scan (m/z range, 150-2000) from 0 to 55 min. Product ion spectra were recorded with a collision energy of 30 eV except for metabolites AR-C133913 and M1, M2, M11, and M12, where a collision energy of 25 eV was used. The mobile phase was degassed before use. Chromatographic separations were performed on a Zorbax SB C18 column (500 × 4.6 mm i.d., 5 μm; Agilent Technologies, Santa Clara, CA) protected by a precolumn (12.5 × 4.6 mm i.d.) with the same packing material. For the analysis of mouse fecal extracts, approximately 0.7 ml/min of the LC eluent was diverted to a radiochemical detector for online LSC. The radiochemical detector was a Radiomatic TR 500 (PerkinElmer Life and Analytical Sciences) equipped with a 500-μl flow-cell for homogeneous counting and operated under the control of the Flo-One software package. The analysis was performed at a target ratio of 3:1, liquid scintillation fluid to mobile phase. To monitor the radioactivity in the mouse urine samples, a flow-cell counter was used. Approximately 0.7 ml/min was transferred for fraction collection in Deep-Well LumaPlate microplates (PerkinElmer Life and Analytical Sciences) using a fraction collector (FC204; Gilson, Inc., Middleton, WI) with a fraction collection interval of 9 s. The Deep-Well LumaPlate microplates, containing approximately 105 μl per well, were allowed to dry in a ventilated area at room temperature. The microplates were then closed by a sealing film (TopSeal A; PerkinElmer Life and Analytical Sciences) and placed in a microplate scintillation counter (TopCount; PerkinElmer Life and Analytical Sciences). The counting results for each of the samples were stored as ASCII files and then exported into Microsoft Excel where peak integration was performed.

LC-MS/MS system 3 consisted of a LC system (series 1100; Agilent Technologies), and the mass spectrometer used was a hybrid quadrupole time-of-flight mass spectrometer (Micromass Q-ToF 2; Waters, Milford, MA) with an electrospray interface and a LockSpray probe. Chromatographic separations were performed on a Zorbax SB C18 column (150 × 4.6 mm i.d., 5 μm; Agilent Technologies) protected by a precolumn (12.5 × 4.6 mm i.d.) with the same packing material. The LC eluent was split, and approximately 0.9 ml/min was transferred for fraction collection into Deep-Well LumaPlate microplates (PerkinElmer Life and Analytical Sciences) using a fraction collector (FC204; Gilson, Inc., Middleton, WI) with a fraction collection interval of 0.17 min (10.2 s). The microplates were allowed to dry in a ventilated area at room temperature and were then closed with sealing film (TopSeal A; PerkinElmer Life and Analytical Sciences) and placed in a microplate scintillation counter (TopCount; PerkinElmer Life and Analytical Sciences). The counting results for each of the samples were stored as ASCII files and then exported into Microsoft Excel where peak integration was performed.

LC-MS/MS gradient methods. For all LC-MS/MS systems, the mobile phase consisted of two solvents, A (0.1% formic acid in water) and B (acetonitrile).

### Table 2 — Continued.

<table>
<thead>
<tr>
<th>Drug or Metabolites</th>
<th>m/z (M+H)+</th>
<th>Major Fragment Ions</th>
<th>Proposed Structures</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>M13</td>
<td>537</td>
<td>509, 491, 467, 449, 415, 373, 335, 293</td>
<td><img src="image" alt="Proposed Structures" /></td>
<td>Mice: MLM; Rat: milk, feces, RH, RLM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Marmosets: urine, feces, MMLM; Dog: DH, DLM; Cyno monkey: CMLM; Human: HH, HLM</td>
</tr>
<tr>
<td>M14</td>
<td>685</td>
<td>523</td>
<td></td>
<td>M13 537 509, 491, 467, 449, 415, 373, 335, 293</td>
</tr>
</tbody>
</table>

**Notes:**
- MLM, mouse liver microsomes; RH, rat hepatocytes; RLM, rat liver microsomes; MMLM, marmoset liver microsomes; DH, dog hepatocytes; DLM, dog liver microsomes; CMLM, cyno monkey liver microsomes; HH, human hepatocytes; HLM, human liver microsomes.
- Human in vivo data were from literature (Teng et al., 2010) and were included here for comparison only.
- Exact location was unknown.
- Tentative assignment.
Two slightly different gradient methods were used in these systems. LC-MS/MS gradient method 1 was used for all in vitro samples and for plasma, milk, and feces samples from all species, whereas gradient method 2 was used for urine samples only to separate polar metabolites. In LC-MS/MS gradient method 1, solvent B started at 5%, held for 1 min, and linearly increased to 35% at 5 min, held at 35% for 20 min, increased to 50% at 40 min, then increased to 95% at 45 min, held at 95% for 3 min, then decreased to 5% at 48.1 min, and held for 7 min until next injection. In LC-MS/MS gradient method 2, solvent B started at 5%, held for 1 min, and linearly increased to 15% at 15 min, increased to 35% at 25 min, then increased to 50% at 40 min, continued to increase to 95% at 45 min, held at 95% for 3 min, then decreased to 5% at 48.1 min, and held for 7 min until next injection.

Results

Excretion of [14C]Ticagrelor in Mice, Rats, and Marmosets

In the excretion mass balance studies in mice, rats, and marmoset monkeys, the excretion of [14C]ticagrelor-associated radioactivity was monitored for a total of 3, 5, and 7 days, respectively. The recovery values of radioactive dose in urine and feces during the entire collection period are shown in Table 1. In each species, the total recovery of radioactive dose was >80%, indicating good mass balance (Roffey et al., 2007). Fecal excretion was the major route of elimination of drug-related radioactivity in all species studied (ranging from 59% in marmoset monkeys to 96% in mice). Less than 5% of the administered radioactivity was excreted via the urine in mice and rats, although renal excretion was more significant in marmosets (7–15%). Excretion was rapid in mice and rats for both oral and intravenous administration, with the majority of the administered dose eliminated in excreta by 24 h after dose (>90% for mice and >75% for rats). Excretion was relatively slow in marmoset, with approximately 44 to 61% of the administered dose recovered in excreta by 24 h after dose. However, in each species, the routes and rates of excretion were similar between both sexes and both routes of administration.

Metabolite Profiles in Plasma, Milk, Urine, and Feces

The presence of ticagrelor metabolites in the various matrices examined and their identities are listed in Table 2. Representative HPLC radiochromatograms from pooled plasma, urine, and feces after oral administration of [14C]ticagrelor in rats are shown in Fig. 2. Similar profiles were observed in the corresponding matrices for mice and marmoset (figures not shown). Because most of the metabolites in the urine samples were much polar in nature, a modified HPLC method was developed to resolve the peaks observed. The identities of ticagrelor and the metabolites in different matrices were verified by LC-MS/MS together with radioactivity detection.

Plasma. The relative distribution of radioactive metabolites in representative plasma samples after oral administration of [14C]ticagrelor is summarized in Table 3. The metabolite profiles of ticagrelor after intravenous dose are similar to those observed following oral dose in mice, rats, and marmosets (data not shown). The recovery of radioactivity in extracted plasma samples is generally good (80–90%). Unchanged ticagrelor was identified as the main component in plasma samples across all species for both oral and intravenous doses. The major metabolites detected in all preclinical animal plasma samples were M5 and M8, which matched authentic synthetic standards for AR-C124910 (loss of the hydroxyethyl side chain) and AR-C133913 (loss of the difluorophenylcyclopropyl group) (structures shown in Fig. 1). Metabolite profiling was only carried out by HPLC with radiodetection and comparison to reference standards for mouse plasma samples. For rat and marmoset plasma samples, additional metabolite characterization was carried out using LC-MS/MS, and a few minor metabolites were detected besides the major components described above, which included M4a (a glucuronide metabolite of AR-C124910), M9a (a glucuronide metabolite of ticagrelor), M10a (a hydroxylated metabolite of ticagrelor), and M12 (loss of the hydroxyethyl side chain of AR-C133913).

Urine. The relative distribution of radioactive metabolites in urine after oral administration of [14C]ticagrelor is summarized in Table 4. The metabolite profiles of ticagrelor after intravenous dose are similar to those observed after oral dose in mice, rats, and marmosets (data not shown). The extraction efficiency after centrifugation was generally 85% or better across all species. Ticagrelor and major circulating metabolite M8 were not detected in the mouse and rat urine samples but were detected in the marmoset urine samples at very low levels. Metabolite M5 was the major metabolite detected in the rat and marmoset urine samples. Besides M5, the metabolites accounting for more than 1% of the dose were M1 in male mouse urine and M12 in marmoset urine, both probably derived from metabolite M5. Most of the minor metabolites detected were secondary metabolites derived from metabolite M5.

Feces. The relative distribution of radioactive metabolites in urine after oral administration of [14C]ticagrelor is summarized in Table 5. The metabolite profiles of ticagrelor after intravenous dose are similar to those observed after oral dose in mice, rats, and marmosets (data not shown). The radioactivity extracted from repeated extraction was generally 80% or better across all species. Substantial amounts of ticagrelor were detected in fecal extracts and accounted for most of the radioactivity found in feces in all species, approximately 33, 42, and 27% of the dose in mouse, rat, and marmoset feces, respectively. Metabolites M5 and M8 were the major metabolites found in fecal extracts from all species. Multiple further hydroxylated metabolites of M8 were also found across species, with M7a and M7c being the dominant ones in mouse feces, whereas M7d and M7e were more abundant in rat and marmoset feces. Likewise, several further hydroxylated metabolites of ticagrelor at various positions were also detected and excreted in feces, where M10b appeared to be the most abundant metabolite observed in all species. A carboxylic acid metabolite, M13, was found as a major component in rat and marmoset feces, accounting for approximately 4.6 and 7.7% of total dose in marmoset and rat feces, respectively. This metabolite was not detected in mouse feces.

Milk. The mean concentrations of total radioactivity in lactating rat milk and plasma are listed in Table 6. At all time points, the mean concentration of total radioactivity in milk was higher than in plasma. Metabolite characterization was also carried out to lactating rat milk samples. The majority of the radioactivity (60–79%) found in lactating rat milk samples was due to unchanged ticagrelor. The distribution of ticagrelor and its metabolites characterized in lactating rat milk is listed in Table 7. The major metabolite was M8, accounting for 9 to 13% of the total radioactivity. Metabolite M5 was also a significant metabolite detected in lactating rat milk samples and accounted for 1 to 13% of the total radioactivity. Other minor metabolites detected were M7a (hydroxylated AR-C124910 metabolite), M10a and M10b (hydroxylated metabolites), M14 (a glucose conjugate), and M13 (a carboxylic acid metabolite). Each of those minor metabolites accounted for less than 4% of the total radioactivity excreted in milk.

Metabolite Comparison In Vitro. In general, the majority of the radioactivity was recovered in the sample supernatants after protein precipitation (~90% or better).

Heptocytes. Metabolite distributions of ticagrelor in rat, dog, and human hepatocytes are shown in Table 8. After a 4-h incubation, ticagrelor remained as the most abundant component in all species (38–69%). M5, M8, and the carboxylic acid metabolite M13 were...
detected as major metabolites in all species examined. Other minor metabolites detected were further hydroxylated metabolites of M8 and ticagrelor. In human hepatocytes, glucuronide conjugates of ticagrelor (M9a) and M8 (M6b) were detected. Both of these metabolites were also detected in dog hepatocyte samples. M9a was also found in rat hepatocyte samples.

Microsomes. Metabolite profiles of ticagrelor after a 1-h incubation with ticagrelor in mouse, rat, marmoset, dog, cynomolgus monkey, and human liver microsomes are shown in Table 9. The turnover rate of ticagrelor appeared relatively slow in incubations with rat, marmoset, dog, and human liver microsomes, with more than 82% of the parent remaining after 1 h. The metabolism of ticagrelor in incubations of cynomolgus monkey and mouse liver microsomes was more extensive (40–55% of parent remaining) relative to the other species tested. Metabolites M5, M8, and M13 were detected in all samples as the major components. Other minor metabolites detected were further hydroxylated metabolites of M8 and ticagrelor. Metabolite profiles were qualitatively similar across species, although quantitative differences were observed. No human specific metabolite was detected in hepatocytes or microsomes.

Characterization of Metabolites. Metabolites were characterized by LC-MS/MS analyses of extracts from plasma, urine, and feces as well as in vitro samples. Characterization of metabolite profiles of ticagrelor by LC-MS/MS was also performed on lactating rat milk extracts obtained from in vivo rat studies. The proposed prominent in vivo metabolic pathways of ticagrelor are shown in Fig. 3. The
proposed structures of \([^{14}C]\text{ticagrelor}\) metabolites from in vitro and in vivo samples are shown in Table 2.

**Ticagrelor.** A typical LC-MS/MS product ion spectrum of ticagrelor obtained in the positive ion mode is shown in Fig. 4. Ticagrelor showed a molecular ion \([M + H]^+\) at \(m/z\) 523. The major fragment ion at \(m/z\) 495 resulted from neutral loss of \(N_2\) from the triazopyrimidine ring. Other characteristic fragment ions were \(m/z\) 477 (loss of water from \(m/z\) 495), \(m/z\) 453 (loss of \(S\)-propyl side chain from \(m/z\) 495), \(m/z\) 453 (loss of both water and \(S\)-propyl side chain), and \(m/z\) 335 (loss of 5-(2-hydroxyethoxy)-cyclopentan-1,2-diol moiety from \(m/z\) 495).

Metabolite M5 (AR-C133913). Metabolite M5 matched with synthetic standard AR-C133913 by retention time and mass fragmentation patterns. It gave a molecular ion \([M + H]^+\) at \(m/z\) 479 and characteristic fragment ions of \(m/z\) 451 (loss of \(N_2\)), \(m/z\) 415 (loss of two water molecules from \(m/z\) 451), \(m/z\) 409 (loss of \(S\)-propyl side chain from \(m/z\) 451), \(m/z\) 373 (loss of \(S\)-propyl side chain from \(m/z\) 415), and \(m/z\) 363 (loss of 5-hydroxy-cyclopentan-1,2-diol moiety from \(m/z\) 479). Metabolite M8 is formed presumably via O-dealkylation to lose the difluorophenylcyclopropyl group from the parent molecule. Metabolite M8 matched with synthetic standard AR-C133913 by retention time and mass fragmentation patterns. It gave a molecular ion \([M + H]^+\) at \(m/z\) 479 and characteristic fragment ions of \(m/z\) 451 (loss of \(N_2\)), \(m/z\) 415 (loss of two water molecules from \(m/z\) 451), \(m/z\) 409 (loss of \(S\)-propyl side chain from \(m/z\) 451), \(m/z\) 373 (loss of \(S\)-propyl side chain from \(m/z\) 415), and \(m/z\) 363 (loss of 5-hydroxy-cyclopentan-1,2-diol moiety from \(m/z\) 479). Metabolite M8 is formed presumably via O-dealkylation to lose the hydroxethyl side chain from the parent molecule.

**Metabolites M1 and M2.** M1 and M2 showed a molecular ion \([M + H]^+\) at \(m/z\) 387, which was 16 atomic mass units (amu) higher than M5. Both M1 and M2 are proposed as hydroxylated M5 with hydroxylation occurring at different locations. M1 and M2 gave different mass fragmentation patterns. The most predominant fragment ion of M1 was \(m/z\) 345 (loss of \(S\)-propyl side chain), followed by \(m/z\) 327 (loss of water from \(m/z\) 345) and \(m/z\) 317 (neutral loss of \(N_2\) from \(m/z\) 345). The observation of \(m/z\) 345 ion suggested that the \(S\)-propyl side chain was intact and, therefore, hydroxylation is proposed to occur in a region other than the \(S\)-propyl side chain for M1. In contrast, the major fragment ion of M2 was \(m/z\) 369 (loss of water), followed by \(m/z\) 341 (loss of \(N_2\)) and \(m/z\) 351 (loss of water). Unlike the parent or M5, there was no loss of \(S\)-propyl side chain observed. The data suggested that the hydroxyl group was at the \(S\)-propyl side chain for M2, most likely as a secondary alcohol metabolite resulting from the facile neutral loss of water.

**Metabolite M3a.** M3a showed a molecular ion \([M + H]^+\) at \(m/z\) 503 with a major fragment ion of \(m/z\) 327 (loss of 176 amu) and is...
proposed to be a glucuronide conjugate of M12. The exact location of glucuronide conjugation could not be determined.

Metabolite M4a. M4a showed a molecular ion of [M+H]+ at m/z 547, 176 amu higher than M5 (AR-C133913). The major fragment ion was m/z 371, consistent with the proposed structure as a glucuronide conjugate of M5 with unknown position of conjugation.

Metabolites M6a and M6b. Metabolites M6a and M6b eluted at different retention times, but both gave a molecular ion of [M+H]+ at m/z 657. The major fragment ion was 479 for both metabolites, i.e., formed via neutral loss of 176 amu, indicating a glucuronide conjugate. Both metabolites are proposed as glucuronide conjugates of M8 (AR-C124910) although the position of conjugation remains unknown.

Metabolites M7a–M7e. M7a–M7e all showed a molecular ion of m/z 477 (loss of water), followed by neutral loss of N2 (m/z 449). This fragmentation pattern suggested that the hydroxylation occurred at the S-propyl side chain, most likely a secondary alcohol metabolite. M7b showed a major fragment ion of m/z 467 (loss of N2), other fragment ions observed were m/z 449 (loss of water from m/z 467), m/z 431 (loss of two water molecules from m/z 467), m/z 409 (loss of hydroxylated S-propyl side chain from m/z 467), m/z 391 (loss of water from m/z 409), and m/z 373 (loss of hydroxylated S-propyl side chain). The observation of m/z 409, m/z 391, and m/z 373 fragment ions all suggested that the hydroxylation occurred at the S-propyl side chain, most likely as a primary alcohol metabolite. M7c gave a major fragment ion of m/z 453 (loss of S-propyl side chain). These data suggested that the hydroxylation occurred at any position other than the S-propyl side chain. Both M7d and M7e showed a characteristic fragment ion of m/z 327, which corresponded to the loss of the difluoroarylfluoroalkylcyclopropyl moiety. Thus, the hydroxylation is proposed to occur at the difluoroarylfluoroalkylcyclopropyl moiety for both metabolites.

Metabolites M9a and M9b. M9a and M9b showed a molecular ion of [M+H]+ at m/z 699 and gave a major fragment ion of m/z 523. They eluted at different retention times, and M9b was only detected in dog hepatocyte samples. Both metabolites are proposed to be glucuronide conjugates of ticagrelor at unknown positions.

Metabolites M10a–M10d. M10a–M10d all showed a molecular ion of [M+H]+ at m/z 539, which was 16 amu higher than ticagrelor. These were all proposed as hydroxylated ticagrelor isomers with the hydroxyl group at different positions. These metabolites eluted at different retention times. M10a gave a major fragment ion of m/z 511 (loss of N2) with other fragment ions of m/z 493 (loss of water from m/z 511) and m/z 453 (loss of hydroxylated S-propyl side chain from m/z 511). The fragmentation patterns were similar to those observed for M7b. The position of the hydroxyl group is proposed at the S-propyl side chain, most likely as a primary alcohol metabolite. M10b showed a major fragment ion of m/z 521 (loss of water), followed by a characteristic ion of m/z 493 (loss of N2). The fragmentation patterns were similar to those observed for metabolites M7a and M2. Thus, the hydroxylation is proposed to occur at the S-propyl side chain, most likely as a secondary alcohol metabolite. M10c and M10d both gave a major fragment ion of m/z 511 (loss of N2) and characteristic fragment ions of m/z 371 (loss of hydroxylated difluoroalkylcyclopropyl group) and m/z 343 (loss of N2 from m/z 371). The hydroxylation is proposed to occur at the difluoroalkylcyclopropyl moiety for both metabolites.

Metabolite M11. M11 showed a molecular ion of [M+H]+ at m/z 343 with a major m/z 301 fragment ion (loss of S-propyl side chain, or 16 amu higher than metabolite M12). This metabolite is proposed to be a hydroxylated M12 with the hydroxyl group most likely at the pyrimidine moiety.

Metabolite M12. M12 showed a molecular ion of [M+H]+ at m/z 327. Fragment ions observed were m/z 299 (loss of N2), m/z 285 (loss of S-propyl side chain), m/z 267 (loss of both water and S-propyl side chain), m/z 263 (loss of 2 water molecules), and m/z 257 (loss of both...
N₂ and S-propyl side chain). It is proposed to be a metabolite formed by loss of both the hydroxyethyl side chain and the difluorophenyl- cyclopropyl moiety from ticagrelor.

**Metabolite M13.** M13 gave a molecular ion of [M+H]⁺ at m/z 537, or 14 amu higher than ticagrelor. The most predominant fragment ion was m/z 509 (loss of N₂). Other characteristic fragment ions were m/z 467 (loss of S-propyl side chain from m/z 509), m/z 449 (loss of water from m/z 467), m/z 415 (loss of two water molecules from m/z 509), and m/z 335 (loss of oxidized cyclopentane-1,2-diol moiety). In addition, this metabolite eluted at the same retention time as the parent molecule under the acidic LC conditions (pH ~2), supporting a carboxylic acid moiety in this metabolite. Based on the HPLC retention time and MS fragmentation patterns, M13 is proposed to be a carboxylic acid metabolite resulting from oxidation of the 2-hydroxyethoxy side chain attached to the cyclopentane-1,2-diol group.

**Metabolite M14.** M14 was only observed in lactating rat milk samples and showed a molecular ion of [M+H]⁺ at m/z 685 with a product ion at m/z 523. Based on the molecular weight and fragmentation patterns, it is proposed as a glucose conjugate of ticagrelor with an unknown position of conjugation.

**Discussion**

Ticagrelor is an oral, direct-acting antiplatelet agent. This study reports the disposition and metabolism of [¹⁴C]ticagrelor in mice, rats, and marmosets with comparison to metabolite profiles found in human. The metabolite profiles from in vitro systems in human and preclinical species are also reported here for comparison. The in vivo metabolism studies reported here were conducted in the animal species used for toxicity testing of ticagrelor. The oral dose (20 mg/kg) level represented the lowest dose used in preclinical toxicity studies. Our findings showed that ticagrelor is metabolized extensively in these species studied, with a total of 21 metabolites characterized across species from both in vitro and in vivo matrices.

The routes of excretion of total administered radioactivity were similar in both sexes and after oral or intravenous administration in mice, rats, and marmosets. Fecal excretion of ticagrelor-related radioactivity was the primary route of elimination (59–96%), suggesting biliary elimination. Urinary excretion was a minor elimination pathway in preclinical species (1–15%). In comparison, approximately 27 and 58% of the dose was found in urine and feces in humans (Teng et al., 2010). The preclinical mass balance data were in good agreement with human data. The excretion patterns in human seemed to be more similar to those observed in marmoset monkeys compared to those in mice and rats.

The guidance recommends that metabolites that are present in quantities greater than 10% of the parent systemic exposure in humans (Food and Drug Administration) or 10% of total drug-related exposure (ICH), as determined by the area under the curve (AUC) values, be exposed after parent drug administration in at least one of the preclinical toxicology species at approximately equivalent or greater quantities, to avoid discrete safety testing. The major circulating metabolites in plasma samples including analytical methods using LC-MS/MS were developed for the determination of ticagrelor and metabolites in plasma samples including preclinical toxicology species at approximately equivalent or greater quantities, to avoid discrete safety testing. The major circulating metabolites in plasma after ticagrelor administration in preclinical species and in a human absorption, distribution, metabolism, and excretion study were identified as ticagrelor and its active metabolite AR-C124910 (Teng et al., 2010). Therefore, rapid and sensitive analytical methods using LC-MS/MS were developed for the determination of ticagrelor and metabolites in plasma samples including preclinical toxicology species (Sillén et al., 2010). Multiple dosing data in healthy volunteers demonstrated predictable pharmacokinetics of ticagrelor and its metabolite over the dose range of 50 to 600 mg once daily and 50 to 300 mg twice daily with C_{max} and AUC_{0-1} increasing approximately dose-proportionally (Butler and Teng, 2010). The calculated AUC_{0-24h} of AR-C124910 was approximately 3400 ng·h/mL at day 5 after twice daily dosing of 100 mg (equivalent to the recommended efficacious dose of 90 mg b.i.d.) (Butler and Teng, 2010). Preclinical studies evaluating the toxicology of ticagrelor in mice, rats, and marmosets showed that exposures to ticagrelor and AR-C124910 in these models exceeded those observed in humans. For example, the exposure of AR-C124910 (AUC_{0-24h}) was approximately 7600 ng·h/mL in male rats at day 31 after 1-mg/kg daily dosing of ticagrelor (AstraZeneca; data on file). Therefore, although AR-C124910 are present in quantities greater than 10% of the parent systemic exposure in humans, or 10% of total drug-related exposure, the human metabolite exposure was well covered in the preclinical toxicity species.

After oral administration of [¹⁴C]ticagrelor to lactating rats, milk samples displayed significantly higher levels of total radioactivity than plasma at all time points. This demonstrates that ticagrelor and/or its metabolites were readily transferred into rat maternal milk; therefore, neonatal rats could be exposed to ticagrelor-related compounds via maternal milk. Ticagrelor was the major component (60–79% of total radioactivity in the samples), whereas metabolites M8 and M5 accounted for the majority of the remaining radioactivity (10–25% total) in lactating rat milk. Drug-related material can be excreted into milk via passive diffusion and/or active secretion, determined by many factors (Ito and Lee, 2003). Several recent studies suggested breast cancer resistance protein involvement in the concentration of drugs into milk as one of the mechanisms (Jonker et al., 2005; van Herwaarden and Schinkel, 2006). There are no current data to suggest which mechanism accounts for the observation of excretion of ticagrelor-related material into rat milk samples. In addition, the excretion of ticagrelor and/or its metabolites in human milk has not been investigated yet.

Metabolite AR-C133913 was one of the major metabolites detected in urine samples across all species. Other metabolites in urine included hydroxylated M5 (M1 and M2), M12, and hydroxylated M12 (M11). Metabolite M12 was a secondary metabolite of M5 formed via the elimination of the hydroxyethyl side chain. These results were consistent with human absorption, distribution, metabolism, and excretion data in which M5 was the major component and both M1 and M2 were detected in human urine (Teng et al., 2010). Although M12 was not detected in human urine, its glucuronide conjugate M3 (1.9% of dose) was reported (Teng et al., 2010). A glucuronide conjugate of

![Diagram of metabolites and fragmentation schemes](image-url)
M12 (M3a) was also detected in marmoset urine at low levels (0.1% of dose). The exact position of the glucuronide conjugation was not determined in these studies, and based on mass fragmentation data alone, it cannot be concluded that these conjugates have the exact same identity. Another significant metabolite detected in human urine was M4 (6.6% of dose), proposed to be a glucuronide conjugate of M5 (AR-C133913) (Teng et al., 2010). A glucuronide conjugate of M5 (M4a) was also detected in marmoset urine samples (0.5% of dose). However, based on mass fragmentation data alone, no definite conclusion can be drawn that these two conjugates were identical.

Ticagrelor, AR-C124910, and AR-C133913 were the major components in feces samples from mice, rats, and marmosets. Multiple hydroxylated AR-C124910 (M7a–M7c) and ticagrelor (M10a–M10d) metabolites were observed. In human, other than ticagrelor, AR-C124910, and AR-C133913, the only other fecal metabolite detected was M7, a hydroxylated AR-C124910 metabolite. The reported mass fragmentation patterns of M7 (Teng et al., 2010) matched M7a detected in this study. A very low level metabolite, M10, was also reported in human urine (Teng et al., 2010), which was proposed as a hydroxylated metabolite of ticagrelor. Because of the low abundance and limited data, it was difficult to conclude which hydroxylated metabolites (M10a–M10d) from this study matched the one observed in human.

In vitro, metabolism of ticagrelor seemed to be less extensive, particularly in microsomes, than that observed in vivo with mostly parent remaining after a 60-min incubation. The major metabolites observed in all in vitro systems investigated were AR-C124910 and AR-C133913, which is consistent with the observation in vivo. Formation of AR-C124910 is proposed via O-dealkylation to lose the hydroxethyl side chain. Loss of the difluorophenylcyclopropyl group via N-dealkylation results in the formation of M5. In vitro experiments with human liver microsomes have shown that ticagrelor is primarily metabolized by CYP3A enzymes (Zhou et al., 2011). CYP3A4 and CYP3A5 seemed to be the enzymes mainly responsible for the formation of AR-C124910, whereas formation of metabolite AR-C133913 was most likely by CYP3A4, with less contribution from CYP3A5 (Zhou et al., 2011). Glucuronides of AR-C124910 (M6a and M6b) were detected in dog and/or human hepatocytes, with only a trace amount of glucuronides of AR-C124910 (M6) reported in human urine (Teng et al., 2010). In the in vitro metabolism pathways were qualitatively similar across species. Most of the metabolites formed in vitro were also observed in vivo. However, more extensive metabolite profiles were observed in vivo than those seen in vitro with hepatocytes and microsomes in terms of relative abundance and secondary metabolism of ticagrelor and AR-C124910. For example, M1 and M2 have been detected in mice, rat, marmosets and human urine samples, but in vitro, they were only detected in cynomolgus monkey microsomes incubate. In addition, several minor metabolites were only detected from in vivo systems (e.g., M3a, M4a, M7d, M7e, M10a, M10c, M10d, M11, and M14).

In summary, these studies demonstrated that metabolic pathways of ticagrelor in the animal species used for toxicological evaluations were qualitatively similar to those seen in human. AR-C124910 and AR-C133913 were the major metabolites observed in all species. Ticagrelor together with its active metabolite AR-C124910 were the most prominent circulating drug-related components in mice, rats, and marmosets. The metabolism and elimination of ticagrelor in preclinical species seem to be mediated primarily by oxidative metabolism and excretion in feces.

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Authorship Contributions
Participated in research design: Li, Landqvist, and Grimm.
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Performed data analysis: Li and Landqvist.
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References

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