Pharmacokinetics of Vicagrel, a Promising Analog of Clopidogrel, in Rats and Beagle Dogs

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ABSTRACT: The objective of this investigation was to compare the efficiency of conversion to the active metabolite (AM) from clopidogrel and vicagrel, a novel antiplatelet agent, and support the drug design rationale in the view of the pharmacokinetics. Following intravenous administration to rats, vicagrel was rapidly converted to its thiolactone intermediate (2-oxo-clopidogrel), then to the AM. The transformation efficiency of vicagrel to 2-oxo-clopidogrel was 94%, but only 13% of clopidogrel was converted to 2-oxo-clopidogrel. Compared with the clopidogrel following oral administration to rats and beagle dogs at equal molar doses, vicagrel increased the exposure to 2-oxo-clopidogrel approximately sixfold (58.6 ± 10.2 vs. 10.2 ± 6.6 μg h/L in rats, 97.1 ± 51.9 vs. 16.1 ± 3.3 μg h/L in dogs) and the exposure to the AM approximately fourfold to sixfold (59.0 ± 18.8 vs. 14.4 ± 9.6 μg h/L in rats, 635.1 ± 114.5 vs. 99.0 ± 10.3 μg h/L in dogs). The rapid and extensive conversion of vicagrel to the intermediate 2-oxo-clopidogrel by esterase instead of cytochrome P450s (CYPs) makes the novel prodrug vicagrel a promising agent to prevent platelet aggregation and overcome clopidogrel resistance and high interindividual variability due to CYP2C19 polymorphism. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:741–749, 2013

Keywords: vicagrel; clopidogrel; prodrugs; 2-oxo-clopidogrel; drug resistance; active metabolite; pharmacokinetics; polymorphism; drug metabolizing enzymes

INTRODUCTION
Currently, platelet aggregation and activation were the main cause leading to acute coronary syndromes and other thrombotic complications.1,2 Clopidogrel is the most widely used antiplatelet agent to prevent coronary artery disease, peripheral vascular disease, and cerebrovascular disease.3,4 Clopidogrel in combination with aspirin is the gold standard prescription to treat patients undergoing percutaneous coronary interventions.5,6 However, in recent clinical practice, interindividual variability in the pharmacodynamic response of clopidogrel has emerged frequently with about 20%—40% patients being classified as nonresponders, low-responders, and clopidogrel resistant.7–9 In most instances, clopidogrel resistance was associated with loss-of-function mutations of CYPs that mediate the two-step sequential CYP metabolism of clopidogrel to the active metabolite (AM).10 AM then covalently binds to the P2Y12 receptor and irreversibly inhibits ADP-induced platelet aggregation.5,11 Reduction in AM formation may contribute to the low- or non pharmacodynamic response.12,13

Among ethnic populations, common polymorphisms of CYP2C19 occur in 30% of whites, 40% of black, and 55% of Asian.14,15 Moreover, CYP2C19 is involved in both steps of CYP-dependent biotransformation of clopidogrel and substantially contributes to the formation of the AM.16 Genetic variants of CYP2C19 are the major determinant among common clopidogrel resistance.17 How to overcome clopidogrel resistance is an urgent issue in the clinical practice, especially for the treatment of the poor metabolizer carrying CYP loss-of-function variants.15,18 Unfortunately, there is no effective way to overcome the low- or nonresponse to clopidogrel. Increasing daily dose of clopidogrel and applying a new generation
of thienopyridine such as prasugrel have not been successful as these approaches may be accompanied with fatal bleeding risk or lack of compliance, or other problems.\textsuperscript{19,20} It is challenging to maintain the balance of efficacy and safety.

Considering the fact of the world-wide prescription and long-term use of clopidogrel, as well as its common resistance,\textsuperscript{21} a novel agent designed on the main skeleton of clopidogrel to overcome clopidogrel resistance would be desired. On the basis of this rationale, a novel ester prodrug (chemical structure shown in Figure 1), vicagrel was synthesized and was expected to be hydrolyzed into its thiolactone intermediate 2-oxo-clopidogrel via esterase instead of CYPs,\textsuperscript{22} as shown in Figure 2. Therefore, vicagrel is believed to have faster onset of action because of its extensive hydrolysis to the intermediate. Vicagrel was designed to reduce the clopidogrel resistance and improve the safety by decreasing the unexpected bleeding. For the inhibition of ADP-induced platelet aggregation in rats, vicagrel exhibited more potent antiplatelet aggregation activity than clopidogrel, and was slightly less potent than prasugrel. Incubated in the rat liver microsomes with the presence of nicotinamide adenine dinucleotide phosphate and glutathione, vicagrel was converted to its AM, identical to that of clopidogrel.\textsuperscript{22} Therefore, the novel agent is believed to be promising with enhanced pharmacodynamic response than clopidogrel and reduced possibility of bleeding risk under a manageable and proper dose range.\textsuperscript{22}

The present study was to compare the efficiency of the formation of thiolactone intermediate and the AM of clopidogrel and vicagrel in rats and beagle dogs following oral (p.o.) or intravenous (i.v.) administration, and to validate the rationale of the drug design with pharmacokinetics (PK) data.

**EXPERIMENTAL**

**Materials**

Vicagrel (free base), 2-oxo-clopidogrel, and clopidogrel (hydrogen sulfate) were kindly offered by State Key Laboratory of Natural Medicines and Center of Drug Discovery, College of Pharmacy, China Pharmaceutical University (Nanjing, China) with a purity of 98\%, 99\%, and 99\%, respectively, and the chemical structures were shown in Figure 1. MP-AM (AM derivatized by reaction with 3-methoxyphenacyl bromide, MPBr) was synthesized by the Center of Drug Discovery, China Pharmaceutical University. R-95913 (prasugrel thiolactone metabolite) with purity greater than 98\% was supplied by Chia-Tai Tian Qing Pharmaceutical Company Ltd. (Jiangsu, China). MPBr was purchased from TCI Company Ltd. (Tokyo, Japan). Acetonitrile and methanol of high-performance liquid chromatography (HPLC) grade were purchased from Tedia (Fairfield, Ohio). Sodium carboxymethyl cellulose (CMC-Na) was kindly offered by Dai-Ichi Kogyo Seiyaku Company Ltd. (Shanghai Branch, China). Formic acid, acetic acid, N, N-dimethylacetamide (DMA), polyethylene glycol (PEG) 400, and other chemicals were of analytical grade.

**Animals**

The present study was approved by the Animal Ethics Committee of China Pharmaceutical University. Sprague–Dawley rats (200 ± 20 g) of both genders were purchased from Shanghai SIPPR/BK Experimental Animal Company Ltd. (Shanghai, China). Beagle dogs (10.0 ± 0.5 kg) of both genders were supplied by Southeast University Laboratory Animal Center (Nanjing, China). Animals were kept under controlled conditions with temperature maintained at 20 ± 2\°C and relative humidity at 50 ± 20\%, and acclimatized to the housing environment for 1 week before the study. The rats and beagle dogs were fasted but provided free access to water for overnight before the administration.

**Preparation of Dosing Form**

Before the administration, the stability of prepared dosage formulations in CMC-Na or DMA plus PEG400 was evaluated. At room temperature, vicagrel (set at 1.14 mg/mL) in both prepared solutions remained stable up to 6 h (data not shown). Therefore, for the i.v. administration to rats, vicagrel-free base, 2-oxo-clopidogrel, and clopidogrel were dissolved in DMA (5% of final volume) and PEG (95% of final volume) solution, with a final concentration at 3 μmol/mL. For p.o. administration to rats and beagle dogs, vicagrel and clopidogrel were prepared in CMC-Na suspensions at a final concentration at 1.8 and 2.0 mg/mL for rats, 5 and 5.4 mg/mL for beagle...
Figure 2. Proposed metabolic pathways of vicagrel and clopidogrel (upper); the derivatization of active metabolite (lower).

dogs, respectively. The dosing solutions were prepared freshly before use.

Derivatization Efficiency of AM
It is believed that clopidogrel AM is unstable in biological fluids because of the thiol group. MPBr has been used as an effective derivatizing reagent to stabilize the AM. The derivatization efficiency of AM was vigorously evaluated by adding different amount of MPB into plasma samples harvested following dose administration. Under optimized derivatization temperature and time (room temperature, 10 min), formation of MP-AM increased with the increment of MPBr concentration from 0 to 5 mM, and remained at steady from 5 to 10 mM. The reaction was depicted in Figure 2. Therefore, excessive amount of MPBr at 10 mM final concentration was used. AM was quantified as MP-AM (graph not shown).

Thiolactone (2-Oxo-Clopidogrel) Exposure Following i.v. Administration to Rats
Vicagrel, 2-oxo-clopidogrel, or clopidogrel dissolved in DMA plus PEG400 was i.v. administrated to rats via caudal vein at a dose of 10 μmol/kg. About
100 μL blood was collected from retinal venous plexus into heparinized tubes predose and postdose at 5, 10, 20, 30, 60, 120, 180, 240, 360, and 720 min, and centrifuged at 12,000 g for 60 s at 4°C immediately. The harvested plasma was immediately pipetted to a triple volume of acetonitrile containing 50 ng/mL R-95913 (internal standard, ISTD), 10 mM MPBr, and 5% acetic acid (terminal concentration) to prevent possible degradation of metabolites and derivatize AM. The prepared samples were stored at −70°C till analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The conversion efficiency of vicagrel and clopidogrel to 2-oxo-clopidogrel was evaluated by comparing the area under the curve (AUC) up to the last time point (AUC\(_{0,t}\)) of 2-oxo-clopidogrel following i.v. administration of vicagrel or clopidogrel with that following i.v. administration of an equal molar dose of 2-oxo-clopidogrel (AUC\(_{2-oxo-clopidogrel}\) from vicagrel/AUC\(_{2-oxo-clopidogrel}\) from clopidogrel or AUC\(_{2-oxo-clopidogrel}\) from vicagrel/AUC\(_{2-oxo-clopidogrel}\) from clopidogrel). Furthermore, the biotransformation rate constant (\(k_t\)) of vicagrel or clopidogrel to 2-oxo-clopidogrel and were calculated using the method established by Yang et al. The rate constant was calculated by the equation \(\frac{dC_1}{dt} = k_{21} \cdot C_1 - (k_{12} + k_1) \cdot C_1\), where \(C_1\) was the concentrations of clopidogrel or vicagrel in central compartment, \(k_{21}\) was the rate constant distributed from central to peripheral compartment, \(k_{12}\) was the rate constant distributed from peripheral to central compartment, and \(k_t\) was the rate constant defined as the transformation rate constant from clopidogrel or vicagrel to 2-oxo-clopidogrel. The user-defined mechanistic two-compartment open PK models using the WinNonlin computer program (Version 4.0; Pharsight Corporation, Mountain View, California) was attempted to evaluate the ratios.

Conversion Efficiency of Vicagrel and Clopidogrel Following p.o. Administration

Vicagrel and clopidogrel suspended in CMC-Na were p.o. administered to rats (50 μmol/kg) and beagle dogs (19.3 μmol/kg). About 1 mL blood was collected from beagle dogs via forearm vein and 100 μL blood collected from rat retinal venous plexus into heparinized tubes at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 720 min. The collected samples were processed as mentioned above, and AM was derivatized under the optimized conditions (as shown in Fig. 2). The AM exposure was used to compare the conversion efficiency of vicagrel and clopidogrel following p.o. administration.

LC–MS/MS Assay

The derivatized AM and 2-oxo-clopidogrel were analyzed simultaneously by a validated LC–MS/MS method. The LC–MS/MS system consisted of an API4000 and an HPLC system. The HPLC system included a LC20AD binary pump system, a SIL20AD autosampler and a CTO 20A oven (Shimadzu, Kyoto, Japan). The analytical column was Shimadzu Shim-pack VP-ODS (150 × 2.0 mm\(^2\)), coupled with a Security Guard C18 guard column (4 × 3.0 mm\(^2\); Phenomenex, Torrance, California). The mobile phase consisted of water containing 0.1% formic acid and 5 mM ammonium acetate (mobile phase A) and acetonitrile (mobile phase B) with a flow rate set at 0.35 mL/min. The autosampler temperature was kept at 4°C and 5 μL of sample solution was injected. The analytes were separated by gradient elution. The initial composition was 10% (mobile phase B) for 1.0 min, then increased to 90% in 0.5 min, and maintained at 90% from 1.5 to 5 min, followed by change to the initial condition in 0.5 min and reequilibrated at 10% (mobile phase B) for 1.0 min. Each run time was 6.5 min. The temperature of the column was set at 25°C. Mass spectra were acquired by the API 4000 MS/MS system equipped with electrospray ionization interface with Turbo Spray ion source. The ion spray voltage was set at 5500 V and the temperature was maintained at 200°C. The nebulizing gas was high purity nitrogen, and Gas 1 and Gas 2 were set at 20. Curtain gas and collision gas (CAD) were 10 and 5, respectively.

Quantification was performed in multiple reaction monitoring (MRM) mode with a dwell time of 0.2 s for each transition. The transitions monitored were fragments of parent ions at m/z 338.2, 504, and 332.1 to their corresponding product ions at m/z 155.1, 212.1, and 149.1, under the collision energy set at 35, 25.4, and 35 eV, and declustering potential set at 45, 36.5, and 45 for 2-oxo-clopidogrel, MP-AM, and R-95913 (ISTD), respectively. The peak area ratio of drug to internal standard, and the concentrations were calculated by Analyst software (version 1.5.1, AB SCIEX, Framingham, MA, USA).

PK Analysis

A noncompartmental model was used to analyze individual time–concentration profiles of 2-oxo-clopidogrel and AM. The maximum plasma concentration (\(C_{\text{max}}\)) and the time of peak concentration (\(T_{\text{max}}\)) were directly derived from the observed data. Area under curve (AUC\(_{0,t}\)) and AUC up to infinity (AUC\(_{0,\infty}\)) were estimated by the trapezoidal rule. The total plasma clearance (CL) following i.v. administration was calculated as dose/AUC\(_{0,\infty}\), where the dose for the formed 2-oxo-clopidogrel was adjusted by the conversion efficiency of vicagrel or clopidogrel. The metabolite ratio was the exposure ratio between AM and 2-oxo-clopidogrel, which was introduced to evaluate the possibility of presystemic metabolism of 2-oxo-clopidogrel by comparison of i.v. and intragastric administration (AUC\(_{\text{AM}}\)/AUC\(_{2-oxo-clopidogrel}\)). The PK parameters were calculated by WinNonlin computer.
program (Version 4.0; Pharsight Corporation). The plasma concentration–time profiles were established by scientific plotting and data analysis software Origin (Version 8.0; Origin Lab, Wheeling, Illinois).

RESULTS

Method Validation

An eight-point calibration curve was established with the linearity range from 0.78 to 100 ng/mL for 2-oxo-clopidogrel, and 1.8 to 500 ng/mL for MP-AM. The calibration curves were constructed by plotting the peak area ratios between the analytes and ISTD with weighted \(1/x^2\) least squares linear regression. The deviation between the nominal concentration and measured concentration was generally less than 15%. The lowest limit of quantification was 0.78 and 1.8 ng/mL for 2-oxo-clopidogrel and MP-AM, respectively.

The recoveries (%) of QC samples at low, middle, high level after treating with acetic acid (5% of the total volume of the sample) were 104.05 ± 1.68, 103.09 ± 5.00, and 103.56 ± 1.10 for 2-oxo-clopidogrel and 103.41 ± 5.14, 102.05 ± 3.21, 102.72 ± 1.15 for MP-AM, respectively, by comparison with the nominal concentration. Meanwhile, there was no significant matrix effect observed for 2-oxo-clopidogrel, MP-AM or ISTD.

2-Oxo-clopidogrel and MP-AM remained stable up to 12 h at 4°C in autosampler, and 15 days in −70°C storage with less than 15% deviation from nominal concentrations for all three QC levels.

The samples that exceeded the maximum limit of quantitation were diluted to the linear range of the standard curve. The QC samples were diluted by the same level as the plasma sample with the blank matrix to evaluate the reliability. The accuracy of the diluted three QC samples was within 15% deviation from nominal concentration.

Conversion Ratios of Vicagrel and Clopidogrel in Rats Following i.v. Administration

The plasma concentration–time profile of 2-oxo-clopidogrel and AM was depicted in Figures 3 and 4. Following i.v. bolus administration of 2-oxo-clopidogrel, vicagrel, or clopidogrel, the AUC\(_{0-t}\) of 2-oxo-clopidogrel was 759.3 ± 103.9, 710.3 ± 65.9, and 81.0 ± 10.7 µg h/L, respectively. Following i.v. dosing of vicagrel, 2-oxo-clopidogrel appeared in circulation immediately, and declined with a half-time (t\(_{1/2}\)) of 0.71 ± 0.10 h. About 94% of vicagrel was cleaved into 2-oxo-clopidogrel after the systemic exposure to vicagrel by comparing the AUC of 2-oxo-clopidogrel from vicagrel and 2-oxo-clopidogrel itself. However, only 13% of clopidogrel was converted to 2-oxo-clopidogrel (AUC\(_{2-oxo-clopidogrel}\) from clopidogrel/AUC\(_{2-oxo-clopidogrel}\)) and peaked at 27 min, with a transformation rate constant fitted at 0.091 min\(^{-1}\) calculated by the method mentioned above (Fig. 3). The mean retention time (MRT), CL and distribution volume (V) of 2-oxo-clopidogrel are all summarized in Table 1.

Conversion of Vicagrel and Clopidogrel Following p.o. Administration to Rats and Beagle Dogs

After p.o. administration of vicagrel to rats, AUC\(_{0-t}\), C\(_{max}\), T\(_{max}\) of 2-oxo-clopidogrel were 58.6 ± 10.2 µg h/L, 21.3 ± 10.1 µg/L, and 0.45 ± 0.2 h, respectively. AUC\(_{0-t}\), C\(_{max}\), and T\(_{max}\) of AM were 10.2 ± 6.6 µg h/L, 10.9 ± 7.9 µg/L, and 0.9 ± 0.7 h, respectively, following p.o. administration of clopidogrel to rats, as shown in Figures 5 and 6. The PK parameters are summarized in Table 2. Following p.o. administration of vicagrel, the metabolite ratio between AM and
Table 1. Pharmacokinetic Parameters of 2-Oxo-Clopidogrel and AM Following Vicagrel, 2-Oxo-Clopidogrel, and Clopidogrel Intravenous Administration at 10 μmol/kg to Rats

<table>
<thead>
<tr>
<th></th>
<th>2-Oxo-Clopidogrel</th>
<th>2-Oxo-Clopidogrel</th>
<th>Vicagrel</th>
<th>Clopidogrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (μg/L)</td>
<td>1295 ± 175</td>
<td>1255 ± 5</td>
<td>48.7 ± 4.0</td>
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<tr>
<td>T_max (min)</td>
<td>5</td>
<td>5</td>
<td>27 ± 6</td>
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<tr>
<td>AUC(0–t) (μg h/L)</td>
<td>759.3 ± 103.9</td>
<td>710.3 ± 65.9</td>
<td>810.0 ± 10.7</td>
<td></td>
</tr>
<tr>
<td>AUC(0–∞) (μg h/L)</td>
<td>762.4 ± 103.4</td>
<td>712.7 ± 65.8</td>
<td>827.3 ± 10.6</td>
<td></td>
</tr>
<tr>
<td>MRT(0–t) (h)</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>t_1/2 (h)</td>
<td>1.2 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>V (L/kg)</td>
<td>5.5 ± 1.15</td>
<td>5.45 ± 1.2</td>
<td>6.12 ± 1.41</td>
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</table>

2-oxo-clopidogrel was 0.90, but less than 0.10 compared with that following i.v. administration. AUC_{0–t} values of 2-oxo-clopidogrel and AM were also used to compare the efficiency between vicagrel and clopidogrel administration to beagle dogs. The plasma concentration versus time profiles of 2-oxo-clopidogrel and AM were depicted in Figures 7 and 8. As shown in Table 2, the AUC_{0–t} values of 2-oxo-clopidogrel of vicagrel and clopidogrel were 97.1 ± 51.9 and 16.1 ± 3.3 μg h/L, whereas those of AM were 635.1 ± 114.5 and 99.0 ± 10.3 μg h/L, respectively. The t_1/2 of 2-oxo-clopidogrel and AM from vicagrel were similar to those from clopidogrel in rats and beagle dogs following p.o. administration. Following p.o. administration of vicagrel, both the AUC_{0–t} of 2-oxo-clopidogrel and AM were about fourfold to sixfold higher than that following clopidogrel at 50 μmol/kg for rats and 24 μmol/kg to beagle dogs, respectively.

Figure 5. Plasma concentration (mean ± SD, n = 5) of 2-oxo-clopidogrel following intragastric administration of vicagrel and clopidogrel at 50 μmol/kg to rats.

Figure 6. Plasma concentration (mean ± SD, n = 5) of AM following intragastric administration vicagrel and clopidogrel at 50 μmol/kg to rats.

Figure 7. Plasma concentration (mean ± SD, n = 4) of 2-oxo-clopidogrel following intragastric administration vicagrel and clopidogrel at 24 μmol/kg to beagle dogs.
Table 2. Pharmacokinetic Parameters of 2-Oxo-Clopidogrel and AM Following Intragastric Administration of Vicagrel and Clopidogrel at 50 μmol/kg to Rats and 24 μmol/kg to Beagle Dogs

<table>
<thead>
<tr>
<th></th>
<th>Vicagrel</th>
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<th>Clopidogrel</th>
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<tr>
<td></td>
<td>Rats</td>
<td>Beagles</td>
<td>Rats</td>
<td>Beagles</td>
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<tr>
<td>2-Oxo-Clopidogrel</td>
<td></td>
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</tr>
<tr>
<td>$C_{\text{max}}$ (μg/L)</td>
<td>21.3 ± 10.1</td>
<td>58.8 ± 18.2</td>
<td>10.9 ± 7.9</td>
<td>16.5 ± 1.9</td>
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<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>0.9 ± 0.7</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>AUC$_{(0-t)}$ (μg h/L)</td>
<td>58.6 ± 10.2</td>
<td>97.1 ± 51.9</td>
<td>10.2 ± 6.6</td>
<td>16.1 ± 3.3</td>
</tr>
<tr>
<td>AUC$_{(\infty)}$ (μg h/L)</td>
<td>63.1 ± 11.5</td>
<td>97.5 ± 52.1</td>
<td>10.7 ± 5.3</td>
<td>17.4 ± 3.1</td>
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<tr>
<td>MRT$_{(0-t)}$ (h)</td>
<td>3.4 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>2.4 ± 0.8</td>
<td>1.0 ± 0.4</td>
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<tr>
<td>$t_{1/2}$ (h)</td>
<td>3.1 ± 1.1</td>
<td>0.8 ± 0.4</td>
<td>2.2 ± 1.8</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>AM</td>
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<tr>
<td>$C_{\text{max}}$ (μg/L)</td>
<td>36.2 ± 15.4</td>
<td>569.1 ± 291</td>
<td>9.6 ± 4.9</td>
<td>107.4 ± 74.3</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.7 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>AUC$_{(0-t)}$ (μg h/L)</td>
<td>59.0 ± 18.8</td>
<td>635.1 ± 114.5</td>
<td>14.4 ± 9.6</td>
<td>99.0 ± 10.3</td>
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<tr>
<td>AUC$_{(\infty)}$ (μg h/L)</td>
<td>61.0 ± 19.2</td>
<td>640.0 ± 111.9</td>
<td>14.9 ± 10.1</td>
<td>99.5 ± 4.4</td>
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<tr>
<td>MRT$_{(0-t)}$ (h)</td>
<td>1.9 ± 0.5</td>
<td>0.9 ± 0.1</td>
<td>1.8 ± 0.9</td>
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<tr>
<td>$t_{1/2}$ (h)</td>
<td>1.8 ± 1.2</td>
<td>1.0 ± 0.3</td>
<td>2.3 ± 2.0</td>
<td>1.2 ± 0.4</td>
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</table>

Figure 8. Plasma concentration (mean ± SD, n = 4) of AM following intragastric administration vicagrel and clopidogrel at 24 μmol/kg to beagle dogs.

DISCUSSION

In our study, a simplified and optimized derivatization method was established to determine the concentrations of AM in plasma. For the ester prodrug vicagrel, we evaluated the extent of the biotransformation to the correspondent metabolites in rats and beagle dogs, in comparison with clopidogrel.

Following i.v. administration to rats, vicagrel was hydrolyzed so rapidly that plasma concentration of vicagrel could not be quantified. Conversion of clopidogrel to 2-oxo-clopidogrel was slower with a $t_{1/2}$ of 7 min (calculated from transformation rate constant $k_1$, $t_{1/2} = 0.693/k_1$). As an ester prodrug of clopidogrel, vicagrel exhibited rapid onset and high efficiency similar to prasugrel.22 The AM exposure was above fivefold higher following i.v. vicagrel administration than clopidogrel, as 94% of vicagrel was hydrolyzed but only 13% of clopidogrel was metabolized to 2-oxo-

clopidogrel. Rapid and efficient conversion of vicagrel to the 2-oxo-clopidogrel intermediate is probably the contributor to the approximately fivefold increase in 2-oxo-clopidogrel and AM exposure in rats and dogs following p.o. administration of vicagrel as compared with p.o. administration of clopidogrel at the same molar dose. The CL and distribution volume (V) of the formed 2-oxo-clopidogrel from vicagrel were calculated by an “effective dose” of 2-oxo-clopidogrel multiplying the dose of vicagrel by the conversion percentage. CL and V were similar to those following i.v. administration of 2-oxo-clopidogrel, and exceeded the hepatic blood flow and the total body liquid in rats, respectively. Moreover, plasma concentrations of AM were much higher than that of 2-oxo-clopidogrel after p.o. dosing of vicagrel and clopidogrel in beagle dogs (Figs. 7 and 8). But plasma concentrations of AM and 2-oxi-clopidogrel in rats were similar levels (Figs. 5 and 6). 2-Oxo-clopidogrel may be metabolized into AM more likely in beagle dogs than in rats after the formation of 2-oxo-clopidogrel from vicagrel and clopidogrel. In rats, the formed 2-oxo-clopidogrel may be inclined to the hydrolysis pathway, which is a comparative pathway to the formation of AM.26,27 However, in beagle dogs, the CYP-dependent AM formation pathway may be the dominant pathway by compared with the hydrolysis pathway. The difference may be related to the different esterase activity in rats and beagle dogs.28,29 The esterase activity in rat intestine is higher than that in beagle dogs, where there is almost no esterase or esterase-like activity in the intestine.30

For clopidogrel, it was reported that about 85% was hydrolyzed in vivo to inactive carboxylic acid metabolite; only a small proportion of clopidogrel was bioactivated sequentially to the AM by CYPs such as CYP2C19, CYP1A2, and CYP2B6 in the first step leading to the formation of thiolactone, and CYP3A4,
2B6, 2C19, 2C9 in the second step leading to formation of the AM.\textsuperscript{31–33} And CYPs polymorphism was related to the most cases of clopidogrel resistance. However, for vicagrel, except the hydrolysis process, the formation of AM from vicagrel shared the same pathway as clopidogrel from 2-oxo-clopidogrel. The ester prodrug design increased the formation efficacy and amount of 2-oxo-clopidogrel from vicagrel. Because of the pre-systemic hydrolysis of vicagrel and rapid formation of 2-oxo-clopidogrel by esterase other than polymorphic CYPs, vicagrel could overcome clopidogrel resistance related to CYP polymorphisms and reduce the interindividual variability. Following p.o. administration, vicagrel was probably hydrolyzed during the absorption from the intestinal tract due to the high expression of esterase in the intestine,\textsuperscript{28} and the hydrolysis of vicagrel probably takes place within epithelial cell. After that hydrolysis, its correspondent metabolite 2-oxo-clopidogrel was metabolized subsequently into AM mediated by the CYP isoforms in the intestine. CYP3A may substantially contribute to the bioactivation of thiolactone intermediate to AM.\textsuperscript{34} Therefore, the various types of esterase in blood, kidney, liver, intestine, and other tissues may contribute to the rapid cleavage of vicagrel to its thiolactone intermediate.

**CONCLUSIONS**

A novel antiplatelet agent, vicagrel was extensively and rapidly converted to 2-oxo-clopidogrel and AM, with about five fold higher exposure than clopidogrel in rats and beagle dogs at equal molar doses. The novel prodrug could be a promising agent to prevent platelet aggregation and overcome clopidogrel resistance and high interindividual variability due to CYP2C19 polymorphism.

**REFERENCE**


