Gender Difference in the Pharmacokinetic Interaction between Oral Warfarin and Oxolamine in Rats: Inhibition of CYP2B1 by Oxolamine in Male Rats

Xuan Zhua,b, Dae Y. Leea and Wan G. Shin*a

a College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea
b Pharmaceutical Department, Medical College, Xiamen University, XiaMen City, FuJian Province, China

ABSTRACT: The possible reason for the significantly greater AUC of oral warfarin with oral oxolamine in male Sprague–Dawley rats was evaluated. After oral administration of warfarin at a dose of 2 mg/kg to male rats with oxolamine at doses of 10 and 50 mg/kg, the AUC values of warfarin were significantly greater than the controls (254 and 330 versus 180 µg h/ml). However, the AUC values of warfarin were not affected by oxolamine in female rats. This could be due to inhibition of CYP2B1, 2C11 and 3A2 by oxolamine in male rats, since warfarin was metabolized via CYP1A1, 2B1, 2C6, 2C11 and 3A2 in rats and CYP2B1 is male dominant, and CYP2C11 and 3A2 are male specific. Therefore, phenytoin, torasemide and clarithromycin (mainly metabolized via CYP2B1/2, 2C11 and 3A2 in rats, respectively) were administered intravenously to male rats with or without oral oxolamine. After oral oxolamine at doses of 10 and 50 mg/kg, the AUC of phenytoin was significantly greater (1280 and 1640 versus 938 µg min/ml), however, the AUC values of torasemide and clarithromycin were independent of oxolamine. The above data suggest that the significantly greater AUC of oral warfarin with oral oxolamine could be due to inhibition of CYP2B1/2 by oxolamine in male rats. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: pharmacokinetic interaction; warfarin; oxolamine; CYP2B1; rats

Introduction

Gender differences in the pharmacokinetics of drugs could be due to the differences in the following factors: the gastric emptying rate, intestinal transit time, gut enzyme, body water space, muscle mass, organ blood flow rate, organ function, body fat and hepatic metabolism, between men and women [1]. Among the factors, differences in the hepatic microsomal cytochrome P450 (CYP) systems play a significant role in the gender differences [1].

Warfarin is widely used as a coumarin anticoagulant with a narrow therapeutic index requiring a tight control in dosage regimen.Commercially available warfarin is a racemic mixture of (R)- and (S)-enantiomers. The racemic warfarin is metabolized via the CYP1A2, 2C9 and 3A4 in humans [2], and the CYP1A1, 2B1, 2C6, 2C11 and 3A2 in rats [3]. It was reported that rat CYP1A1, 1A2, 2B1, 2C11 and 3A1 and human CYP1A1, 1A2, 2B6, 2C9 and 3A4 proteins have 78%, 70%, 74%, 77% and 73% homology, respectively [4]. Hence, the CYP isozymes responsible for the metabolism of racemic warfarin are similar between rats and humans. In this regard, rats could be used as an animal model in order to study pharmacokinetic changes of warfarin. Rat CYP2B1 is male dominant, CYP2C11 and 3A2 are...
male specific, and CYP1A1 and 2C6 have no gender difference [4]. However, studies on the human CYP isozyme do not seem to have been published. In humans, the clearance, volume of distribution, half-life, urinary excretion, plasma protein binding and extent of absolute oral bioavailability ($F$) of warfarin are $0.045 \pm 0.024 \text{ml/min/kg}$, $0.14 \pm 0.061 \text{kg}$, $37 \pm 15 \text{h}$, less than 2%, $99 \pm 1\%$ and $93 \pm 8\%$, respectively [5]. It was reported that there was a gender difference in the pharmacokinetics of oral warfarin in rats; the total area under the plasma concentration–time curve from time zero to time infinity ($AUC$) of oral warfarin at a dose of $2 \text{mg/kg}$ in male Sprague–Dawley rats was significantly smaller (47.8% decrease) than that in female rats [6]. However, a gender difference in the pharmacokinetic parameters of warfarin in humans does not seem to have been published.

Oxolamine [3-phenyl-5-$(\beta$-diethyloaminoethyl)-1,2,4-oxadiazole] citrate is an antiinflammatory agent with a particular antitussive activity. Therefore, it has been used extensively in hospitals for patients with coughs. Oxolamine is also being prescribed to patients who are stable on warfarin therapy. However, the types of CYP isozymes that are inhibited by oxolamine do not seem to have been published yet.

In the anticoagulation services (ACS) in Korean Hospitals (Seoul National University Hospital, Seoul, and Samsung Medical Center, Seoul, Republic of Korea), drug interaction between warfarin and oxolamine has been observed; the international normalized ratio (INR) of warfarin increased in patients coadministered with oxolamine. Similar results were also reported in six male and five female patients [7]. This suggests that the anticoagulant activity of warfarin was increased by oxolamine. Although pharmacokinetic interactions between warfarin and numerous drugs with respect to CYP isozyme changes have been reported [8], a pharmacokinetic interaction between warfarin and oxolamine does not seem to have been published. Hence, rats were used as an animal model in order to find a pharmacokinetic interaction between warfarin and oxolamine in patients.

The purpose of this study was to report the possible reason for the gender difference in the $AUC$ of oral warfarin (significantly greater $AUC$ of oral warfarin coadministered with oral oxolamine to male rats) in terms of possible inhibition of warfarin metabolism by oxolamine via CYP2B1/2 in male rats.

Materials and methods

Chemicals

Oxolamine citrate and torasemide were donated from Dongsung Pharmaceutical Company (Seoul, Republic of Korea) and Roche Pharmaceutical Company (Manheim, Germany), respectively. Warfarin, 7-ethoxycoumarin (an internal standard for high-performance liquid chromatographic, HPLC, analysis of warfarin), tris(hydroxymethyl) aminomethane (Tris$^\text{HCl}$)-buffer, phenytoin and chlorozoxazone (an internal standard for HPLC analysis of torasemide and phenytoin) were purchased from Sigma–Aldrich Corporation (St Louis, MO). Clarithromycin and roxithromycin (an internal standard of HPLC analysis of clarithromycin) were supplied from Research Laboratory of Dong-A Pharmaceutical Company (Yongin, Republic of Korea). Other chemicals were of reagent grade or HPLC grade.

Animals

Male (weighing 250–320 g) and female (weighing 190–260 g) Sprague–Dawley rats of 6–8 weeks of age were purchased from Samtako Bio Korea (Seoul, Republic of Korea). All rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Republic of Korea) at a temperature of between 20 and 23°C with a 12 h light (0700–1900) and dark (1900–0700) cycle and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under a supply of filtered pathogen-free air with food (Samyang Company, Pyeongtaek, Republic of Korea) and water ad libitum. They were fasted overnight before the experiment. The protocol of the animal study was approved by Animal Care and Use Committee of College of Pharmacy of Seoul National University.

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Oral administration of warfarin to male and female rats pretreated with or without oxolamine

Oxolamine citrate (dissolved in distilled water) at doses of 10 and 50 mg/kg as the oxolamine base were administered orally (oral volume of 5 ml/kg) using a feeding tube for 3 consecutive days (3 times per day) to male and female rats. The same volume of distilled water was administered to control rats.

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) were similar to previously reported methods [9]. The carotid artery was cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) under light ether anesthesia on day 4 after the oral administration of oxolamine or distilled water (control rats). The cannula was exteriorized to the dorsal side of the neck, where the cannula was terminated with a long silastic tube (Dow Corning, Midland, MI). The silastic tube was covered with a wire sheath to allow free movement of the rats. They were not restrained during the whole experimental period. A heparinized 0.9% NaCl-injectable solution (approximately 0.3 ml; 15 unit/ml) was used to flush the cannula to prevent blood clotting. Each rat was housed individually in a metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed to recover from anesthesia for 4–5 h before the commencement of the experiment. On day 4 just after oral administration of oxolamine or distilled water, warfarin (dissolved in distilled water) at a dose of 2 mg/kg was administered orally (total oral volume of 1.5 ml) to male rats (n = 10, 10 and 10 for control rats and rats pretreated with oxolamine at doses of 10 and 50 mg/kg, respectively) and female rats (n = 10, 10 and 9 for control rats and rats pretreated with oxolamine at doses of 10 and 50 mg/kg, respectively). An approximately 0.22 ml aliquot of blood sample was collected via the carotid artery in an Eppendorf tube at 0 (to serve as a control), 0.5, 1, 2, 4, 8, 12, 24, 36, 48 and 72 h after oral administration of warfarin. An approximately 0.3 ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 unit/ml) was used to flush each cannula immediately after each blood sampling. Blood samples were centrifuged (HST-8 Centrifuge; Hanil Science Industrial Company, Seoul, Republic of Korea) immediately, and a 100 µl aliquot of each plasma sample was stored in a −70 °C freezer (Model DF8517; Ilshin Laboratory Company, Seoul, Republic of Korea) until HPLC analysis of warfarin [10].

Intravenous administration of phenytoin, torasemide or clarithromycin to male rats pretreated with or without oxolamine

On day 4 just after oral administration of oxolamine, phenytoin (at a dose of 25 mg/kg; dissolved in distilled water with a minimum amount of 10 N NaOH), mainly metabolized via CYP2B1 in rats [11], torasemide (at a dose of 2.5 mg/kg; dissolved in distilled water with a minimum amount of 10 N NaOH and adjusted to a final pH of approximately 8 with Tris-HCl), mainly metabolized via CYP2C11 in rats [12], or clarithromycin (at a dose of 20 mg/kg; dissolved in 0.9% NaCl-injectable solution by adding few drops of 85% phosphoric acid), mainly metabolized via CYP3A2 in rats [13] was infused (total injection volume of 2 ml/kg for each drug) for 1 min via the jugular vein of male rats. The numbers of male rats used were 8, 8 and 7 for control rats and rats pretreated with oxolamine at doses of 10 and 50 mg/kg, respectively, for phenytoin, and the corresponding values for torasemide and clarithromycin were 9, 10 and 10, and 8, 9 and 10. The blood sampling time schedules for phenytoin [11], torasemide [12] and clarithromycin [13] were the same as previously reported methods. The other procedures were similar to the above mentioned warfarin studies.

Measurement of \( V_{\text{max}} \), \( K_m \) and \( \text{Cl}_{\text{int}} \) for the disappearance of warfarin or phenytoin in hepatic microsomal fractions

The procedures are similar to the reported methods [14]. The livers of rats without (control) or with oral oxolamine at doses of 10 and 50 mg/kg (n = 6, 5 and 5 for warfarin and n = 6, 4 and 5 for phenytoin, respectively) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufen, Germany) in an ice-cold buffer of 0.15 M KCl/50 mM Tris-HCl in 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 10,000 × g for 30 min and the supernatant fraction was further centrifuged at 100,000 × g for 90 min. The protein content was measured using the reported
method [15]. The $V_{\text{max}}$ (the maximum velocity) and $K_m$ (the Michaelis–Menten constant; the concentration at which the rate is one-half of $V_{\text{max}}$) for the disappearance of warfarin or phenytoin were determined after incubating the above microsomal fractions (equivalent to 0.5 mg), a 5 μl aliquot of distilled water that contained 1, 5, 10, 50 and 100 μM of warfarin or distilled water with a minimum amount of 10 N NaOH that contained 1, 2.5, 5, 10, 20 and 50 μM of phenytoin and a 50 μl aliquot of Tris-HCl buffer, pH 7.4 that contained 1 mM of NADPH in a final volume of 0.5 ml by adding 0.1 M phosphate buffer, pH 7.4, in a water-bath shaker kept at 37°C and at a rate of 500 oscillations per min (opm). All the above microsomal incubation conditions were linear. The reaction was terminated by the addition of a 1 ml aliquot of diethyl ether after 15 min incubation for phenytoin or 30 min incubation for warfarin. Warfarin [10] or phenytoin [16] was measured by the reported HPLC method. The kinetic constants ($K_m$ and $V_{\text{max}}$) for the disappearance of warfarin or phenytoin were calculated using the nonlinear regression method [17]. The intrinsic clearance ($Cl_{\text{int}}$) for the disappearance of warfarin or phenytoin was calculated by dividing the respective $V_{\text{max}}$ by the respective $K_m$.

**Measurement of plasma protein binding of clarithromycin**

Protein binding of clarithromycin at a concentration of 10 μg/ml with or without oxolamine at a concentration of 10 μg/ml to fresh rat plasma of control rats ($n = 4$, each) was determined using the equilibrium dialysis technique [18]. One ml of plasma was dialysed for 24 h at 37°C against 1 ml of isotonic Sørensen phosphate buffer of pH 7.4 that contained 3% (w/v) dextran in a 1 ml dialysis cell (Spectrum Medical Industries, Los Angeles, CA) using a Spectra/Por 4 membrane (mol. wt cutoff of 12000–14000 Da, Spectrum Medical Industries). After 24 h incubation, two 100 μl aliquots were collected from each compartment and stored in a –70°C freezer until HPLC analysis of clarithromycin [19].

**HPLC analysis of warfarin, phenytoin, torasemide and clarithromycin**

The concentrations of warfarin [10], phenytoin [16], torasemide [12] and clarithromycin [19] in plasma sample were analysed by the reported HPLC methods.

**Pharmacokinetic analysis**

The AUC was calculated using the trapezoidal rule–extrapolation method; this method employs the logarithmic trapezoidal rule for the calculation of the area during the phase of a declining plasma level phase [20] and the linear trapezoidal rule for the phase of a rising level in plasma. The area from the last datum point to the infinity was estimated by dividing the last measured concentration in plasma by the terminal-phase rate constant.

Standard methods [21] were used to calculate the following pharmacokinetic parameters using the noncompartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the first moment of AUC (AUMC), terminal half-life, mean residence time (MRT), apparent volume of distribution at steady state ($V_{\text{ss}}$), and time-averaged total body clearance ($Cl$). The peak plasma concentration ($C_{\text{max}}$) and time to reach a $C_{\text{max}}$ ($T_{\text{max}}$) were read directly from the experimental data.

The harmonic mean method was used to calculate the mean values of $V_{\text{ss}}$ [22], terminal half-life [23] and $Cl$ [24].

**Statistical analysis**

A value of $p < 0.05$ was considered to be statistically significant using a Duncan’s multiple range test of Statistical Package for Social Sciences (SPSS) posteriori analysis of variance (ANOVA) among the three means for the unpaired data or an unpaired t-test. All data are expressed as mean ± standard deviation.

**Results**

**Pharmacokinetics of warfarin after oral administration of warfarin to male and female rats with or without oral oxolamine**

After oral administration of warfarin at a dose of 2 mg/kg to male and female rats pretreated...
with or without oxolamine at doses of 10 and 50 mg/kg, the mean arterial plasma concentrations–time profiles of warfarin are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 1. In male rats, the AUC values of warfarin with oxolamine at doses of 10 and 50 mg/kg were significantly greater (41.1% and 83.3% increase, respectively) than the controls; each value was significantly different. In male rats, the terminal half-lives of warfarin with oxolamine at doses of 10 and 50 mg/kg were significantly longer (50.3% and 75.2% increase, respectively) than the controls; the values were not significantly different between oxolamine at doses of 10 and 50 mg/kg. However, in female rats, the pharmacokinetic parameters of warfarin listed in Table 1 were not significantly different among the three groups of rats.

Table 1. Mean (± standard deviation) pharmacokinetic parameters of warfarin after oral administration of warfarin at a dose of 2 mg/kg without (control) or with oral oxolamine at doses of 10 and 50 mg/kg to male and female rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Oxolamine 10 mg/kg</th>
<th>Oxolamine 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>AUC (µg h/ml)</td>
<td>180 ± 52.0⁴</td>
<td>254 ± 112</td>
<td>330 ± 53.7</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>7.13 ± 3.12</td>
<td>8.42 ± 2.38</td>
<td>8.95 ± 2.61</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>6.50 ± 3.69</td>
<td>6.90 ± 3.60</td>
<td>9.05 ± 10.1</td>
</tr>
<tr>
<td>Terminal half-life (h)</td>
<td>16.1 ± 5.43b</td>
<td>24.2 ± 8.65</td>
<td>28.2 ± 6.89</td>
</tr>
<tr>
<td>Female</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 9)</td>
</tr>
<tr>
<td>AUC (µg h/ml)</td>
<td>345 ± 80.0</td>
<td>395 ± 92.8</td>
<td>333 ± 89.2</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>7.53 ± 1.29</td>
<td>9.66 ± 3.66</td>
<td>7.62 ± 1.17</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>11.6 ± 4.79</td>
<td>13.0 ± 3.66</td>
<td>8.06 ± 6.56</td>
</tr>
<tr>
<td>Terminal half-life (h)</td>
<td>28.4 ± 8.32</td>
<td>24.9 ± 14.1</td>
<td>30.1 ± 7.05</td>
</tr>
</tbody>
</table>

⁴ Each value was significantly different (p < 0.05).
⁵ Control was significantly different (p < 0.05) from oxolamine, 10 and 50 mg/kg.

**Pharmacokinetics of phenytoin, torasemide and clarithromycin with or without oral oxolamine**

After intravenous administration of each drug to male rats pretreated with or without oxolamine, the mean arterial plasma concentrations–time profiles of phenytoin, torasemide and clarithromycin are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration of phenytoin to rats pretreated with oxolamine at a dose of 50 mg/kg, the AUC of phenytoin was significantly greater (74.8% increase) than the controls. After intravenous administration of phenytoin to rats with oxolamine at doses of 10 and 50 mg/kg, the Cl of phenytoin was significantly slower (23.3% and 39.8% decrease, respectively) than
the controls. However, the pharmacokinetic parameters of torasemide listed in Table 2 were not significantly different among the three groups of rats. The pharmacokinetic parameters of clarithromycin listed in Table 2 were also not significantly different among the three groups of rats except for a significantly larger $V_{ss}$ (96.9% increase) and significantly faster $Cl$ (107% increase) with oxolamine at doses of 10 (☐; $n = 8, 10$ and 9 for phenytoin, torasemide and clarithromycin, respectively) or with oxolamine at doses of 50 (⚫; $n = 7, 9$ and 10 for phenytoin, torasemide and clarithromycin, respectively) mg/kg three times per day for 3 consecutive days to male rats. Bars represent standard deviation.

Figure 2. Mean arterial plasma concentration–time profiles of phenytoin (a), torasemide (b) and clarithromycin (c) after intravenous administration at a dose of 25 (phenytoin), 2 (torasemide) and 20 (clarithromycin) mg/kg without (☐; $n = 8, 9$ and 7 for phenytoin, torasemide and clarithromycin, respectively) or with oxolamine at doses of 10 (☐; $n = 8, 10$ and 9 for phenytoin, torasemide and clarithromycin, respectively) or 50 (⚫; $n = 7, 9$ and 10 for phenytoin, torasemide and clarithromycin, respectively) mg/kg three times per day for 3 consecutive days to male rats. Bars represent standard deviation.

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Measurement of $V_{max}$, $K_m$ and $Cl_{int}$ for the disappearance of warfarin or phenytoin in rat liver microsomes

The $V_{max}$, $K_m$ and $Cl_{int}$ for the disappearance of warfarin or phenytoin in hepatic microsomal fractions of the three groups of rats are listed in Table 3. In rats pretreated with oral oxolamine at doses of 10 and 50 mg/kg, the $V_{max}$ for the disappearance of warfarin was considerably slower (24.8% and 32.8% decrease, respectively) and the $V_{max}$ for the disappearance of phenytoin...
Phenytoin

Warfarin

Parameter Control 10 mg/kg 50 mg/kg

Phenytoin (n = 8) (n = 8) (n = 7)

$AUC$ (µg min/ml) 938 ± 182* 1280 ± 327 1640 ± 806

Terminal half-life (min) 39.3 ± 42.1 39.3 ± 32.3 62.4 ± 49.0

$V_{ss}$ (ml/kg) 41.8 ± 24.4 39.5 ± 23.9 60.1 ± 34.1

$Cl$ (ml/min/kg) 806 ± 823 617 ± 694 865 ± 249

Torasemide (n = 9) (n = 10) (n = 10)

$AUC$ (µg min/ml) 1640 ± 441 1670 ± 362 1520 ± 252

Terminal half-life (min) 115 ± 26.0 115 ± 18.5 105 ± 18.8

$V_{ss}$ (ml/kg) 104 ± 22.9 95.7 ± 19.0 94.5 ± 20.5

$Cl$ (ml/min/kg) 1.22 ± 0.301 1.19 ± 0.277 1.32 ± 0.245

Clarithromycin (n = 8) (n = 9) (n = 10)

$AUC$ (µg min/ml) 328 ± 157c 293 ± 58.0 227 ± 61.5

Terminal half-life (min) 121 ± 32.7 121 ± 41.7 147 ± 51.6

$V_{ss}$ (ml/kg) 95.3 ± 20.9 95.1 ± 42.3 106 ± 43.0

$Cl$ (ml/min/kg) 3810 ± 3070b 5590 ± 3890 7500 ± 5740

Table 2. Mean ($±$ standard deviation) pharmacokinetic parameters of phenytoin, torasemide and clarithromycin after intravenous administration at doses of 25, 2, and 20 mg/kg, respectively, without (control) or with oral oxolamine at doses of 10 and 50 mg/kg to male rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>$V_{max}$ (nmol/min/mg protein)</td>
<td>3.16 ± 0.795a</td>
<td>2.37 ± 0.539</td>
<td>2.12 ± 0.852</td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>104 ± 36.5</td>
<td>117 ± 39.4</td>
<td>98.6 ± 26.1</td>
</tr>
<tr>
<td>$Cl_{int}$ (ml/min/mg protein)</td>
<td>0.0312 ± 0.00398b</td>
<td>0.0212 ± 0.00493</td>
<td>0.0219 ± 0.00749</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>(n = 6)</td>
<td>(n = 4)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>$V_{max}$ (nmol/min/mg protein)</td>
<td>1.24 ± 0.493b</td>
<td>0.492 ± 0.507</td>
<td>0.588 ± 0.180</td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>34.1 ± 17.4</td>
<td>20.2 ± 11.0</td>
<td>22.1 ± 6.18</td>
</tr>
<tr>
<td>$Cl_{int}$ (ml/min/mg protein)</td>
<td>0.0396 ± 0.0113b</td>
<td>0.0201 ± 0.014</td>
<td>0.0264 ± 0.00110</td>
</tr>
</tbody>
</table>

*Control was significantly different ($p<0.05$) from oxolamine, 50 mg/kg.  
*bControl was significantly different ($p<0.05$) from oxolamine, 10 and 50 mg/kg.  
*cControl was considerably different ($p<0.1$) from oxolamine, 50 mg/kg.

(60.2% and 52.4% decrease, respectively) was significantly slower than the controls, suggesting that maximum velocity for the disappearance (mainly due to metabolism) of warfarin or phenytoin was slower after oxolamine. However, the $K_m$ values for both the disappearance of warfarin and phenytoin were not significantly different between the three groups of rats, suggesting that the affinity of warfarin or phenytoin to the enzyme(s) was not changed in rats pretreated with oral oxolamine. Hence, the $Cl_{int}$ for the disappearance of warfarin (32.2% and 29.7% decrease for warfarin, respectively) or phenytoin (49.4% and 33.4% decrease, respectively) in rats with oral oxolamine at doses of 10 and 50 mg/kg was significantly slower than the controls. This suggests that in rats pretreated with oxolamine at doses of 10 and 50 mg/kg, the metabolism of warfarin or phenytoin decreased compared with the controls.

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Plasma protein binding of clarithromycin

Plasma protein binding values of clarithromycin with or without oxolamine were 51.9 ± 2.24% and 43.5 ± 4.56%, respectively; they were significantly different (p < 0.05).

Discussion

It was reported that statistically significant correlations between the log pharmacokinetic parameters of warfarin (half-life, metabolic clearance, volume of distribution, intrinsic clearance of unbound drug and unbound volume of distribution of tissues) and the log body weight were obtained from rats and humans [25]. Moreover, the CYP isozymes responsible for the metabolism of racemic warfarin between humans [2] and rats [5] were similar. Hence, rats were chosen as an animal model in the present study.

In male rats, the AUC values of oral warfarin pretreated with oral oxolamine at both doses were significantly greater than the controls (Table 1). This could be supported by significantly slower Cl\text{int} of warfarin by oxolamine (Table 3). However, in female rats, the AUC values of warfarin were not significantly different, regardless of oxolamine pretreatment (Table 1). This gender difference in the changes of AUC values of oral warfarin with oral oxolamine suggests that oxolamine may inhibit CYP2B1, 2C11 and/or 3A2, since warfarin is metabolized via CYP1A1, 2B1, 2C6, 2C11 and 3A2 in rats [2], and CYP2B1 is male dominant, CYP2C11 and 3A2 are male specific, CYP1A2 is female dominant, but CYP1A1 and 2C6 have no gender difference in rats [4].

Hence, phenytoin, torasemide and clarithromycin were intravenously administered to male rats with or without oxolamine to find which CYP isozymes are inhibited by oxolamine in male rats. It was reported that torasemide is mainly metabolized via CYP2C11 in male rats [12]. Hence, torasemide at a dose of 2 mg/kg was administered intravenously to male rats pretreated with oxolamine to find whether oxolamine can inhibit CYP2C11 in rats. The AUC of phenytoin was significantly greater in rats pretreated with oxolamine (Table 2) than the controls, suggesting that the oxolamine can inhibit CYP2B1/2 in male rats. This can be supported by the significantly slower Cl\text{int} in rats pretreated with oxolamine (Table 3).

The gender-different pharmacokinetics of warfarin does not seem to have been published in humans before. It was reported that human CYP2B6 is not male dominant [28].

In conclusion, the gender difference in the significantly greater AUC values of oral warfarin with oral oxolamine in male rats could be due to an inhibition of metabolism of warfarin via CYP2B1/2 by oxolamine.
INHIBITION OF WARFARIN METABOLISM BY OXOLAMINE VIA CYP2B1 IN MALE RATS

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