Evaluation of the Relationship between Sex, Polymorphisms in CYP2C8 and CYP2C9, and Pharmacokinetics of Angiotensin Receptor Blockers

Teresa Cabaleiro, Manuel Román, Dolores Ochoa, María Talegón, Rocío Prieto-Pérez, Aneta Wojnicz, Rosario López-Rodríguez, Jesús Novalbos, and Francisco Abad-Santos

Service of Clinical Pharmacology, Hospital Universitario de la Princesa, Instituto Teofilo Hernando, Instituto de Investigación Sanitaria Princesa, Madrid, Spain (T.C., M.R., D.O., M.T., R.P.-P., A.W., J.N., F.A.-S.); Liver Unit, Gastroenterology Service, Hospital Universitario de La Princesa, Instituto de Investigación Sanitario Princesa, Madrid, Spain (R.L.-R.); and Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain (R.L.-R., F.A.-S.)

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ABSTRACT

Angiotensin II receptor blockers (ARBs) are used to treat hypertension. Most ARBs are metabolized by CYP2C9. The aim of this study is to evaluate the possible association between sex, polymorphisms in the CYP2C8 and CYP2C9 genes, and the pharmacokinetics of losartan, valsartan, candesartan, and telmisartan. The study population comprised 246 healthy volunteers from seven single-dose clinical trials: 64 from two candesartan studies, 43 from a telmisartan study, 36 from a losartan study, and 103 from three valsartan studies. DNA was extracted from blood samples and single-nucleotide polymorphisms in the CYP2C8 (CYP2C8*2, CYP2C8*3, CYP2C8*4, CYP2C8*5) and CYP2C9 (CYP2C9*2, CYP2C9*3) genes were evaluated using real-time polymerase chain reaction. Sex only affected telmisartan pharmacokinetics, since women showed a higher telmisartan Cmax than men (590.5 ± 75.8 ng/ml versus 282.1 ± 30.8 ng/ml; P ≤ 0.01). CYP2C9 variants were associated only with losartan pharmacokinetics: the half-life of losartan was higher in CYP2C9*3 allele carriers (0.1 ± 0.4 hours) than in volunteers with the wild-type genotype (2.3 ± 0.1 hours) (P ≤ 0.05). CYP2C8 polymorphisms were associated only with valsartan pharmacokinetics, since *2 allele carriers showed faster clearance (1.07 ± 0.57 l/h kg) than those with the wild-type genotype (0.48 ± 0.72 l/h kg; P ≤ 0.01) and carriers of the *3 allele (0.35 ± 0.49 l/h kg; P ≤ 0.001). These results suggest that genotypes for CYP2C9 and CYP2C8 are relevant to the pharmacokinetics of losartan and valsartan, respectively, but not the pharmacokinetics of candesartan or telmisartan.

Introduction

Angiotensin II type 1 receptor blockers (ARBs) are used in the treatment of hypertension (Mori et al., 2006). Losartan (Losartan Alter, Cozaar) was the first selective ARB used in the treatment of hypertension (Mori et al., 2006). Losartan (Losartan Alter, Cozaar) was the first selective ARB used in the treatment of hypertension (Mori et al., 2006). Losartan (Losartan Alter, Cozaar) was the first selective ARB used in the treatment of hypertension (Mori et al., 2006). Losartan (Losartan Alter, Cozaar) was the first selective ARB used in the treatment of hypertension (Mori et al., 2006).

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Goa, 1998; Schmidt and Schieffer, 2003; Nakashima et al., 2005). Telmisartan (Telmisartan Alter, Micardis) has no affinity for any of the cytochrome P450 isoenzymes (Unger and Kaschina, 2003), including CYP2C9, and is partially metabolized by glucuronidation (Israel, 2000).

CYP2C8 shares sequence homology with CYP2C9 and can metabolize the same kind of drugs. However, few studies have evaluated the role of CYP2C8 in the metabolism of ARBs (Nakashima et al., 2005).

Point mutations or single-nucleotide polymorphisms in the CYP2C8 and CYP2C9 genes have been identified. The common coding mutations in CYP2C9 are CYP2C9*2, and CYP2C9*3 (Stubbins et al., 1996; Lee et al., 2002). CYP2C9*2 and CYP2C9*3 differ from the wild-type CYP2C9*1 by a single point mutation: CYP2C9*2 is characterized by a 430C>T exchange in exon 3 resulting in an Arg144Cys amino acid substitution, whereas CYP2C9*3 is characterized by a 1075A>C exchange in exon 7 causing an I359L substitution in the catalytic site of the enzyme. Individuals with the *2 or *3 variant alleles could have reduced enzyme activity.

Approximately two-thirds of Caucasians express the wild-type genotype (CYP2C9*1/*1), one-third express either the *1/*2 or *1/*3

ABBREVIATIONS: ARB, angiotensin II receptor blocker; AUC, area under the curve; CI, total drug clearance; Cmax, maximum plasma concentration; C0, last measured concentration; P450, cytochrome P450; k0, apparent terminal elimination rate; PK, pharmacokinetics; t1/2, half-life; T0.5, time to reach Cmax.
The most frequent CYP2C8 variants are *2 (Ile269Phe), *3 (linked polymorphisms Arg139Lys and Lys399Arg), *4 (Ile264Met), and *5 (rare nonsynonymous polymorphic allele). CYP2C8*2, CYP2C8*3, and CYP2C8*4 were associated with reduced enzyme activity (Dai et al., 2001; Daily and Aquilante, 2009; Gao et al., 2010), although some studies have shown higher capacity to metabolize repaglinide, rosiglitazone, pioglitazone, and R-ibuprofen in CYP2C8*3 carriers (Niemi et al., 2003; Kirchheiner et al., 2006; Tornio et al., 2008; López-Rodríguez et al., 2008). Allelic frequencies were 80, 2, 11, 7, and 0% for CYP2C8*1, *2, *3, *4, and *5 in a Spanish population, respectively (López-Rodríguez et al., 2008).

Single-nucleotide polymorphisms in CYP2C8 and CYP2C9 that reduce catalytic activity could modify the clinical benefits of ARBs. Therefore, the aim of this study was to evaluate the possible association between polymorphisms in the CYP2C8 and CYP2C9 genes and the pharmacokinetics of the most commonly used ARBs (losartan, valsartan, candesartan, and telmisartan) in Caucasian individuals. We also evaluated the effect of sex on the metabolism of these drugs.

Materials and Methods

Study Design. Our study population comprised 246 Caucasian healthy adult volunteers enrolled in various bioequivalence single-dose clinical trials performed at the Hospital Universitario de la Princesa (Madrid, Spain) between 2006 and 2012. We genotyped 36 subjects receiving losartan (50 mg), 27 of 36 volunteers participating in a candesartan study (32 mg), 37 of 48 enrolled in a telmisartan study (80 mg), 32 of 36 enrolled in a valsartan study (160 mg), 34 of 54 participating in another valsartan study (320 mg), and 37 of 48 enrolled in a candesartan-hydrochlorothiazide study (25–64). All participants gave their written informed consent for genotyping. These protocols were carried out according to current Spanish legislation on clinical research in humans and were approved by the local clinical investigation ethics committee.

Sample Processing. A peripheral blood sample was taken from each patient and added to a tube with 3 ml of EDTA K3 before being identified with a code and registered. DNA was then extracted from each blood sample in an automatic DNA extractor (MagNa Pure System; Roche Applied Science, Indianapolis, Indiana). The DNA obtained was quantified spectrophotometrically using a NanoDrop ND-1000 Spectrophotometer (Wilmington, DE), and purity was tested using the A260/A280 ratio.

Genotyping. All patients were evaluated for CYP2C8*2, CYP2C8*3, CYP2C8*4, CYP2C8*5, CYP2C9*2, and CYP2C9*3 by real-time polymerase chain reaction in a LightCycler 480 (Roche), as previously described (López-Rodríguez et al., 2008).

Drug Determination. Blood samples were collected for analysis before dosing and at different times after dosing. All blood extractions were carried out using a Venoject system (Terumo Europe N.V.) with sterile EDTA tubes. The tubes were centrifuged at 4°C (at room temperature in the case of losartan) for 10 minutes at 3500 rpm (2330g). Once centrifuged, plasma was aliquoted in two polystyrene tubes and labeled with the protocol code, the subject number, the period, and the time at which the sample was obtained. The aliquots were stored in two different freezers at −70°C (−30°C in the case of losartan and telmisartan) to prevent the possibility of thawing until their transfer to the laboratory responsible for the analytic determinations. The temperatures of these freezers were registered daily during the storage period. Samples were shipped to the laboratory in dry ice. ARBs and internal standards were measured using validated reversed phase high-performance liquid chromatography and detected by tandem mass spectrometry (see Supplemental Material). In the case of losartan, the active metabolite E-3174 was measured in the same subjects that were used for the losartan measurements.

Pharmacokinetics. Pharmacokinetic parameters were estimated from the plasma concentration–time data by noncompartmental analysis with WinNonlin Professional software (version 2.0; Pharsight Corporation). The maximum plasma concentration (Cmax) and time to reach the maximum plasma concentration (Tmax) were obtained directly from raw data. The total area under the curve from administration time to infinity (AUCinf) was calculated as the sum of AUC0→t and the residual area (Ct divided by ke) with Ct as the last measured concentration and ke as the apparent terminal elimination rate, estimated by log-linear regression from the terminal portion of the log-transformed concentration–time plots. The mean of the extrapolated AUC was 2.7% (range, 0.6–8.7). The half-life (t1/2) was calculated by dividing 0.693 by ke.

The total drug clearance adjusted for bioavailability (Cl/F) was calculated by dividing the dose by the AUC and later adjusted for weight (Cl/Fw). In the case of E-3174, total drug clearance was not calculated because we did not know the dose. AUC and Cmax were adjusted for dose and weight and logarithmically transformed for statistical analysis.

Data Analysis. To simplify the analysis and owing to the low number of subjects for some genotypes, we classified CYP2C8 and CYP2C9 genotypes in groups. CYP2C8 genotypes were classified in five groups: the wild-type genotype (*1/*1), allele *2 carriers (including *2/*4 genotype but not *2/*3), allele *3 carriers (including genotypes *2/*3 and *3/*4), and allele *4 carriers (excluding *2/*4 and *3/*4). Since the *5 allele was present in only one subject, it was excluded from the analysis. In the volunteers treated with telmisartan, only one subject had the CYP2C9*2 allele and CYP2C9*4 allele, which were also excluded from the analysis. In the losartan group, only one subject had the CYP2C8*2 allele, which was excluded from the analysis. CYP2C9 genotypes were classified in three groups: the wild-type genotype (*1/*1), allele *2 carriers, and allele *3 carriers (including the *2/*3 genotype).

The Hardy-Weinberg equilibrium was estimated for all analyzed variants. Deviations from the equilibrium were detected by comparing the observed and expected frequencies using a Fisher exact test based on the De Finetti program (available at http://www2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl).

Differences in the genotypic frequencies of variants according to sex were determined using a corrected Pearson χ2 test. The statistical significance of the differences in pharmacokinetic parameters between individuals with different genotypes and sex was calculated using a univariate analysis (t test or analysis of variance). Significant factors were included in a multivariate analysis (multivariate general linear model). SPSS software (version 15; SPSS Inc., Chicago, IL) was used for the statistical analysis. Because this study was designed as an exploratory analysis, we did not adjust P values for multiple testing, which is consistent with prior recommendations (Rothman, 1990; Savitz and Olshan, 1998; Thompson, 1998).

Results

Sample Description and Genotype Frequencies. Sex distribution and age and weight are indicated in Table 1. Weight was higher in men than in women for all of the ARBs studied. CYP2C8 and CYP2C9 genotype frequencies are shown in Table 2; differences in polymorphism distribution between ARBs were established using a χ2 test (P ≤ 0.001). Considering all subjects together, allelic frequencies were 76.0, 15.0, and 8.9% for CYP2C9*1, *2, and *3, respectively, and 76.2, 5.3, 14.2, 4.1, and 0.2% for CYP2C8*1, *2, *3, *4, and *5, respectively. CYP2C9*2 and CYP2C8*3 are well established to be in strong linkage disequilibrium (Shintani et al., 2001; Yasar et al., 2002), and the number of subjects carrying both genotypes is included in Table 2. Actually, 77.4% of subjects carrying CYP2C9*2 allele were also carriers of CYP2C8*3: 83.3% in the losartan study, 85% in the case of candesartan, and 72.2% for valsartan. There were no CYP2C9*2 carriers in the telmisartan study.

Effect of Sex on the Pharmacokinetics of ARBs. On average, the AUC and Cmax of all drugs were higher in women than in men (Table 3); this difference could be attributed to the lower body weight of women (who receive a higher dose by weight). Indeed, most differences disappear after adjusting for dose and weight. Consequently, there were no differences between men and women in the pharmacokinetics of losartan, losartan metabolite E-3174, candesartan, and valsartan. However, women showed a higher telmisartan Cmax.
(adjusted for dose and weight) \( P \leq 0.01 \) (Table 3). Women also showed a higher telmisartan AUC and half-life and a lower clearance, although these differences did not reach statistical significance.  

**Involvement of CYP2C8 and CYP2C9 in the Pharmacokinetics of ARBs.** Pharmacokinetic parameters according to **CYP2C8** and **CYP2C9** polymorphisms are shown in Table 4. No association was found between **CYP2C9** and **CYP2C8** polymorphisms and the pharmacokinetics of candesartan and telmisartan.

Regarding losartan pharmacokinetics, half-life was longer in subjects with the **CYP2C9** wild-type genotype (4.6 hours; 95% CI, 4.4–4.8) than in subjects with the **CYP2C9** wild-type genotype (2.3 hours; 95% CI, 2.0–2.5) \( P \leq 0.05 \). The half-life of the losartan metabolite E-3174 was also higher in subjects with the **CYP2C9** wild-type genotype (5.7 hours; 95% CI, 5.0–6.3) than in subjects with the wild-type genotype (4.6 hours; 95% CI, 4.4–4.8) \( P \leq 0.001 \). The AUC ratio (E-3174 AUC/losartan AUC) was no different between the **CYP2C9** genotypes. In addition, the E-3174 \( C_{\text{max}} \) was lower in subjects with the **CYP2C9** wild-type genotype (4.8 l/h; 95% CI, 4.2–5.4) than in volunteers with the **CYP2C9** wild-type genotype (5.9 l/h; 95% CI, 5.0–6.3) \( P \leq 0.01 \). No differences were observed in the pharmacokinetics of losartan or its metabolite according to **CYP2C8** polymorphisms.

On the other hand, carriers of the **CYP2C8** allele showed higher valsartan clearance (1.07 l/h/kg; 95% CI 0.7–1.5) than subjects with the wild-type genotype (0.48 l/h/kg; 95% CI, 0.3–0.7) \( P \leq 0.001 \). No differences were observed in valsartan pharmacokinetics according to **CYP2C9** polymorphisms. Because **CYP2C9** is linked to **CYP2C8**, in the valsartan study we compared the subjects carrying both **CYP2C9** and **CYP2C8** alleles \( n = 26 \) with the subjects carrying **CYP2C9** allele and **CYP2C8** wild-type allele \( n = 10 \), and we found no differences in pharmacokinetics parameters.

**Discussion**

Drug response depends on several factors, including genetics and sex. ARBs are metabolized mainly by P450 enzymes, although other enzymes may be involved. Polymorphisms in genes coding for these P450 enzymes may explain differences in ARB pharmacokinetics, which can in turn affect drug response. Although telmisartan is not metabolized by these enzymes, we included it to confirm that polymorphisms on P450 enzymes do not affect telmisartan pharmacokinetics.

An association was found between the pharmacokinetics of telmisartan and sex, since \( C_{\text{max}} \) was higher in women than in men. Indeed, the drug label indicates that plasma concentrations of telmisartan are generally 2–3 times higher in women, although there are no significant differences in blood pressure. The difference is due to the slower

### TABLE 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Genotyped</th>
<th>Sex</th>
<th>Participants</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candesartan ( n = 84 )</td>
<td>64</td>
<td>Men</td>
<td>30</td>
<td>25.3 ± 4.3</td>
<td>73.9 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Women</td>
<td>25.8 ± 5.2</td>
<td>60.3 ± 8.3***</td>
<td></td>
</tr>
<tr>
<td>Telmisartan ( n = 48 )</td>
<td>43</td>
<td>Men</td>
<td>19</td>
<td>25.9 ± 5.2</td>
<td>75.4 ± 9.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Women</td>
<td>25.6 ± 5.6</td>
<td>61.0 ± 9.9***</td>
<td></td>
</tr>
<tr>
<td>Losartan ( n = 36 )</td>
<td>36</td>
<td>Men</td>
<td>18</td>
<td>24.7 ± 3.3</td>
<td>76.6 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Women</td>
<td>24.9 ± 4.1</td>
<td>61.3 ± 6.8***</td>
<td></td>
</tr>
<tr>
<td>Valsartan ( n = 138 )</td>
<td>103</td>
<td>Men</td>
<td>49</td>
<td>23.7 ± 3.4</td>
<td>76.7 ± 10.7</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>Women</td>
<td>23.4 ± 2.9</td>
<td>58.7 ± 5.0***</td>
<td></td>
</tr>
</tbody>
</table>

**P < 0.01 versus men in the ARB-matched group; ***P < 0.001 versus men in the ARB-matched group.**

### TABLE 2

**CYP2C8** and **CYP2C9** genotype frequencies for each drug

Table 2: **CYP2C8** and **CYP2C9** genotype frequencies for each drug

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Losartan ( n = 36 )</th>
<th>Candesartan ( n = 64 )</th>
<th>Valsartan ( n = 103 )</th>
<th>Telmisartan ( n = 43 )</th>
<th>All ARBs ( n = 246 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2C8</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>25 (69.4)</td>
<td>35 (54.7)</td>
<td>61 (59.2)</td>
<td>25 (58.1)</td>
<td>146 (59.3)</td>
</tr>
<tr>
<td>*1/*2</td>
<td>1 (2.8)</td>
<td>3 (4.7)</td>
<td>8 (7.8)</td>
<td>9 (20.1)</td>
<td>21 (8.5)</td>
</tr>
<tr>
<td>*1/*3</td>
<td>5 (13.9)</td>
<td>17 (26.6)</td>
<td>23 (22.3)</td>
<td>5 (11.6)</td>
<td>50 (20.3)</td>
</tr>
<tr>
<td>*3/*3</td>
<td>1 (2.8)</td>
<td>1 (1.6)</td>
<td>5 (4.9)</td>
<td>1 (2.3)</td>
<td>8 (3.3)</td>
</tr>
<tr>
<td>*2/*2</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>2 (1.9)</td>
<td>1 (2.3)</td>
<td>11 (4.5)</td>
</tr>
<tr>
<td>*1/*4</td>
<td>3 (8.3)</td>
<td>5 (7.8)</td>
<td>1 (2.9)</td>
<td>0 (0)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>*2/*4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>*3/*3</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>2 (1.9)</td>
<td>0 (0)</td>
<td>3 (1.2)</td>
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<tr>
<td>*4/*4</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>*1/*5</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td><strong>CYP2C9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1</td>
<td>23 (63.9)</td>
<td>31 (48.4)</td>
<td>54 (52.4)</td>
<td>32 (74.4)</td>
<td>140 (56.9)</td>
</tr>
<tr>
<td>*1/*2</td>
<td>6 (16.7)</td>
<td>20 (31.3)</td>
<td>30 (29.1)</td>
<td>1 (2.3)</td>
<td>57 (23.2)</td>
</tr>
<tr>
<td>*2/*2</td>
<td>0 (0)</td>
<td>6 (9.4)</td>
<td>6 (5.8)</td>
<td>0 (0)</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>*1/*3</td>
<td>6 (16.7)</td>
<td>11 (17.2)</td>
<td>10 (9.7)</td>
<td>10 (23.3)</td>
<td>37 (15.0)</td>
</tr>
<tr>
<td>*2/*3</td>
<td>1 (2.8)</td>
<td>1 (1.6)</td>
<td>3 (2.9)</td>
<td>0 (0)</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>*3/*3</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Subjects carrying both <strong>CYP2C8</strong> and <strong>CYP2C9</strong></td>
<td>5 (13.9)</td>
<td>17 (26.6)</td>
<td>26 (25.2)</td>
<td>0 (0)</td>
<td>49 (19.9)**</td>
</tr>
</tbody>
</table>
We did not observe differences in the substrate (tolbutamide) and genotype reported a prolonged half-life in genotypes.

logically active, clinical responses could be similar in the different genotypes. Because E-3174 is pharmaco-
slower metabolism of losartan. E-3174 half-life was also longer in subjects with polymorphisms in this gene could be slower and the CYP2C9*3 allele than in volunteers with the CYP2C9*1/*1 wild-type gene.

by glucuronidation (Israili, 2000; Unger and Kaschina, 2003). No association was found between CYP2C9 and CYP2C8 alleles, and the pharmacokinetics of telmisartan, thus confirming previous findings that this ARB is not metabolized by P450 isoforms, since it has no affinity for any of the P450 isoenzymes and is partially metabolized by glucuronidation (Israili, 2000; Unger and Kaschina, 2003).

As expected, the half-life of losartan was longer in subjects with the CYP2C9*3 allele than in volunteers with the CYP2C9*1/*1 wild-type genotype. Losartan is converted to its metabolite E-3174 mainly by CYP2C9 (Israili, 2000; Yasar et al., 2002); therefore, metabolism in subjects with polymorphisms in this gene could be slower and the half-life of losartan longer, a finding that is consistent with our data. Both in vitro and in vivo investigations suggest that the CYP2C9*2 and/or CYP2C9*3 alleles result in decreased catalytic activity for losartan compared with homozygosity for the *1 allele (Yasar et al., 2001, 2002). The fact that losartan pharmacokinetics is affected by the CYP2C9*3 polymorphism, but not by CYP2C9*2, could be explained because *3 allele codes for an enzyme with lower activity than the one by *2 allele. In this sense, subjects with *2 allele have an intermediate activity phenotype and *3 allele carriers showed a low activity phenotype (Subramanian et al., 2012). In addition, other studies have shown pharmacokinetic associations with CYP2C9*3 but not with CYP2C9*2 (Lee et al., 2012).

The C<sub>max</sub> of E-3174 is lower in CYP2C9*3 carriers because of the slower metabolism of losartan. E-3174 half-life was also longer in carriers of the CYP2C9*3 allele but this can be an artifact because of the slow formation of the metabolite. Because E-3174 is pharmaco-
logically active, clinical responses could be similar in the different genotypes.

A study evaluating the association between another CYP2C9 substrate (tolbutamide) and genotype reported a prolonged half-life in subjects whose genotypes exhibited the *3 allele, but not the *2 allele (Kirchheimer et al., 2002). We did not observe differences in the pharmacokinetics of losartan according to the CYP2C8 polymorphism, possibly reflecting that no losartan metabolite was formed by CYP2C8 in vitro (Babaoglu et al., 2004).

Candesartan cilexetil (cyclohexyl carbonate ester prodrug of candesartan) was identified as a CYP2C8 inhibitor (Walsky et al., 2005); therefore, it is assumed to have affinity for this enzyme. In addition, CYP2C9*3 may change the metabolic activity of candesartan compared with CYP2C9*1 (Hanatani et al., 2001), and the CYP2C9*1/*3 genotype was associated with decreased clearance and increased plasma concentration of candesartan, thus potentially enhancing its hypertensive effect (Uchida et al., 2003). Nevertheless, we did not find any association between the pharmacokinetics of candesartan and any of the polymorphisms studied, maybe because of the low number of subjects and because the effect of CYP2C9 is small, as candesartan is mainly excreted unchanged in urine and feces. Therefore, it is necessary to evaluate this effect in other studies.

Nakashima et al. (2005) studied the association between several P450 enzyme genotypes, including CYP2C8, and valsartan metabolism, and only found involvement of CYP2C9 (Unger and Kaschina, 2003). In addition, no good correlation was observed between the formation rates of 4-OH valsartan and CYP2C8 activity, and CYP2C9 catalyzed 4-hydroxylation of valsartan (Nakashima et al., 2005). However, we found that CYP2C8 variants affect the pharmacokinetics of valsartan, since clearance was higher in subjects carrying the CYP2C8*2 allele than in those with the wild-type genotype. This finding cannot be explained on the basis of current knowledge because CYP2C8*2 was previously shown to be associated with decreased enzyme activity with several CYP2C8 substrates (Dai et al., 2001; Daily and Aquilante, 2009; Gao et al., 2010). The effect of CYP2C8*2 on losartan pharmacokinetics could not be evaluated because there was only one subject carrying this allele, but it did not influence the pharmacokinetics of candesartan or telmisartan.

A potential limitation of our study is that no corrections were made for multiple testing and some false positive results could have been obtained. However, some statisticians recommend never correcting for multiple comparisons while analyzing data (Rothman 1990; Savitz and Olshan 1998; Thompson 1998). They instead recommend reporting all values and making it clear that no mathematical correction was made for multiple comparisons. They recommend accounting for multiple comparisons when interpreting the results rather than in the calculations. It has also been argued that use of multiple testing corrections is an inefficient way to perform empirical research, since multiple testing adjustments control false positives at the potential expense of many more false negatives (Perneger, 1998; Feise, 2002).

### Table 3

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Sex</th>
<th>Losartan</th>
<th>E-3174</th>
<th>Candesartan</th>
<th>Valsartan</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (ng·h/ml)</td>
<td>Men</td>
<td>455.2 ± 42.5</td>
<td>1807.3 ± 573.6</td>
<td>3258.9 ± 692.0</td>
<td>3880.3 ± 1926.2</td>
<td>2469.9 ± 402.6</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>518.8 ± 43.5</td>
<td>2578.4 ± 1758.1</td>
<td>3814.7 ± 910.6</td>
<td>3858.2 ± 2718.1</td>
<td>3677.2 ± 590.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>Men</td>
<td>234.1 ± 23.9</td>
<td>200.0 ± 67.9</td>
<td>216.3 ± 61.6</td>
<td>4592.2 ± 296.1</td>
<td>3821.1 ± 308.8</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>286.5 ± 34.5</td>
<td>301.2 ± 141.4</td>
<td>260.1 ± 63.9</td>
<td>4913.2 ± 294.5</td>
<td>590.5 ± 75.8</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>Men</td>
<td>2.5 ± 0.2</td>
<td>4.9 ± 0.7</td>
<td>11.3 ± 3.3</td>
<td>9.1 ± 0.3</td>
<td>25.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>2.4 ± 0.1</td>
<td>4.8 ± 0.5</td>
<td>12.1 ± 3.7</td>
<td>9.4 ± 0.3</td>
<td>33.1 ± 3.2</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>Men</td>
<td>1.2 ± 0.1</td>
<td>3.7 ± 0.8</td>
<td>5.1 ± 1.3</td>
<td>3.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>1.2 ± 0.1</td>
<td>3.7 ± 0.9</td>
<td>4.9 ± 1.2</td>
<td>3.2 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Cl (l/h/kg)</td>
<td>Men</td>
<td>1.66 ± 0.5</td>
<td>NC</td>
<td>0.14 ± 0.04</td>
<td>0.4 ± 0.5</td>
<td>17.9 ± 14.7</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>1.82 ± 0.7</td>
<td>NC</td>
<td>0.15 ± 0.04</td>
<td>0.6 ± 0.8</td>
<td>11.7 ± 7.7</td>
</tr>
</tbody>
</table>

NC, not calculated.

* P < 0.01 versus men in ARB-matched group.
sex affected the pharmacokinetics of telmisartan.

Another potential limitation is that sample sizes for some of the drugs are relatively small when they are split up into the various genotypes. Therefore, statistical power could be very low to evaluate differences, especially for the rare polymorphisms (CYP2C9*3 or *4 carriers). Therefore, statistical power could be very low to evaluate differences, especially for the rare polymorphisms (CYP2C9*3 or *4 carriers). Therefore, statistical power could be very low to evaluate differences, especially for the rare polymorphisms (CYP2C9*3 or *4 carriers).

Finally, we conclude that the pharmacokinetics of losartan and valsartan, but not of candesartan or telmisartan, is affected by polymorphisms in CYP2C9 and CYP2C8, respectively. In addition, sex affected the pharmacokinetics of telmisartan.

Acknowledgments

This study would not have been possible without the cooperation of the volunteers.

Authorship Contributions

Participated in research design: Ochoa, Novalbos, Abad-Santos.

Conducted experiments: Cabaleiro, Román, Ochoa, Talegón, Prieto-Pérez, Wojnicz, López-Rodríguez, Novalbos, Abad-Santos.

Performed data analysis: Cabaleiro, Román, Ochoa, Abad-Santos.

Wrote or contributed to the writing of the manuscript: Cabaleiro, Román, Ochoa, López-Rodríguez, Abad-Santos.

References


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Address correspondence to: Dr. Teresa Cabaleiro, Service of Clinical Pharmacology, Hospital Universitario de la Princesa, C/Diego de León 62, 28006 Madrid, Spain. Email: teresa.cabaleiro@salud.madrid.org or fabad.hlpr@salud.madrid.org

Pharmacogenetics and Pharmacokinetics of ARBs 229
Supplemental Data: DMD #46292

Title: Evaluation of the relationship between gender, polymorphisms in CYP2C8 and CYP2C9, and pharmacokinetics of angiotensin receptor blockers

Authors: Teresa Cabaleiro, Manuel Román, Dolores Ochoa, María Talegón, Rocio Prieto-Pérez, Aneta Wojnicz, Rosario López-Rodríguez, Jesús Novalbos, Francisco Abad-Santos

Journal Title: DRUG METABOLISM AND DISPOSITION

ARBs quantification by HPLC/MS/MS

Analysis of candesartan was performed by a validated LC/MS/MS method over a concentration range of 1 to 500 ng/ml. The method involved a solid-phase extraction with MCX Oasis plates. Candesartan and internal standard were measured by reversed phase high performance liquid chromatography and detected by tandem mass spectrometry detection (LC/MS/MS). The limit of quantification was 1 ng/ml.

The determination of plasma losartan concentration was performed by a validated LC/MS/MS method over a concentration range of 2 to 800 ng/ml. The method involved a liquid-liquid extraction procedure with tertbutyl methyl ether: 2-propanol procedure. Losartan and internal standard were measured by reversed phase high performance liquid chromatography and detected by tandem mass spectrometry detection (HPLC-MS/MS). The limit of quantification was 2.00 ng/ml.

Analysis of losartan carboxy acid (E-3174) was performed by a validated LC/MS/MS method over a concentration range of 2 to 800 ng/ml. The method involved a liquid-liquid extraction procedure with tertbuthyl methyl ether: 2-propanol (95:5). Losartan carboxy acid and internal standard were measured by reversed phase high performance liquid chromatography and detected by tandem mass spectrometry detection (LC-MS/MS). The limit of quantification was 2.00 ng/ml.

Analysis of valsartan was performed by a validated LC/MS/MS method over a concentration range of 30 to 15000 ng/ml. The method involved a protein precipitation extraction with methanol. Valsartan and internal standard were measured by reversed phase high performance liquid chromatography and detected by tandem mass spectrometry detection (LC/MS/MS).
Chromatographic separations were performed on a reversed-phase column (Zorbax SB-C18, 4.6 x 50 mm, 3.5µm, from Agilent Technologies). The mobile phase was ammonium formate 8 mM pH=3: methanol (30:70 v/v). The chromatographic separation was isocratically performed at room temperature at a flow-rate of 1.00 ml/min. The limit of quantification was 30.06 ng/ml.

Analysis of telmisartan was performed by a validated LC/MS/MS method over a concentration range of 1 to 1500 ng/ml. The method involved a solid-phase extraction procedure with MCX 30 mg Oasis plates. Telmisartan and internal standard were measured by reversed phase high performance liquid chromatography coupled to a tandem mass spectrometry detection (LC/MS/MS). The limit of quantification was 1.00 ng/ml.