Assessment of sex differences in pharmacokinetics and pharmacodynamics of amlodipine in a bioequivalence study

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Abstract

Aims: This study was conducted to assess the bioequivalence between two 10-mg amlodipine tablet formulations. As secondary objectives, sex-related differences and tolerability profile were evaluated.

Methods: Thirty-six healthy volunteers (18 males and 18 females; age 20–32 years, weight 49.5–98.0 kg) were included in a randomised crossover study. Subjects were administered a single 10-mg oral dose of each formulation separated by a 14-day washout period. Plasma amlodipine levels were determined by a high performance liquid chromatographic method with tandem mass spectrometry detection.

Results: All subjects completed the study and 90% confidence intervals for relevant pharmacokinetic parameters were within the ranges defined by European and US Regulatory Authorities: the geometric mean and the 90% confidence interval test/reference ratios calculated from log-transformed values were 104.54 (101.46–107.72%) for AUC0–∞ and 100.32 (97.41–103.33%) for Cmax. There were no serious or severe adverse events. The tolerability profile appeared to be comparable for the two products. On average, bioavailability of amlodipine was slightly higher in females than in males, but these differences could be explained by the lower body weight of women. There were no sex-related differences in drug clearance. Bioequivalence was also demonstrated within each gender group. Amlodipine treatment produced a slight decrease of systolic blood pressure and an increased in heart rate, which were more pronounced in women. The incidence of adverse events was similar in men and women.

Conclusions: The two formulations were considered bioequivalent. Although there were no relevant gender-related differences in the pharmacokinetics of amlodipine, women reached higher amlodipine concentrations most likely because of their lower body weight, and therefore, the reported pharmacodynamic effects were higher within this gender group.

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Keywords: Amlodipine; Calcium channel blockers; Bioequivalence; Healthy volunteers; Pharmacokinetics

1. Introduction

Amlodipine is a widely prescribed dihydropyridine calcium channel-blocking agent structurally related to nifedipine. However, it differs from nifedipine and other dihydropyridines by virtue of its higher oral bioavailability (65%) and longer elimination half-life of 35–50h, enabling once daily dosing [1]. Oral doses of 5–10mg once daily are effective in mild-to-moderate hypertension and stable angina pectoris [1].

Amlodipine is gradually but completely absorbed from the gastrointestinal tract, with peak serum levels occurring within 6–9h. Bioavailability is 60–65% due to first pass metabolism. Maximum plasma concentration (Cmax) is around 3 and 6 ng mL−1 after the administration of single oral doses of 5 and 10mg, respectively [2–5]. The
drug exhibits linear pharmacokinetics [3]. Food does not modify absorption [1], but grapefruit juice might inhibit its metabolism [6], although there is some controversy in this matter [5]. The drug is extensively metabolised in the liver by CYP3A4 to inactive metabolites, with 60% of the dose being excreted in the urine (5–10% unchanged); 20–25% is excreted in faeces. Total protein binding is 93–98%. Amlodipine has a high volume of distribution (about 21 L kg$^{-1}$) and an elimination half-life of 35–50 h in healthy subjects. Clearance is around 0.42 L h$^{-1}$ kg$^{-1}$ [1–8].

This study seeks to carry out a pharmacokinetic analysis of two different amlodipine tablet formulations administered as a single oral dose in healthy volunteers. The primary objective was to demonstrate the bioequivalence of the test formulation compared to the marketed reference formulation according to health authorities criteria [9–11]. As secondary objectives, sex differences in pharmacokinetic parameters and tolerability of the two formulations were evaluated. To the best of our knowledge, no other investigations have looked at pharmacokinetic gender differences for amlodipine.

2. Subjects and methods

The trial was conducted following approval by the Ethics Committee of Hospital de la Princesa (Madrid, Spain), duly authorised by the local regulatory agency, and under the guidelines of Good Clinical Practice. All the subjects gave their written informed consent prior to study enrolment.

2.1. Subjects

Thirty-six healthy volunteers (18 females and 18 males) participated in this study. This sample size was calculated assuming a coefficient of variation for the area under the curve (AUC) and maximum plasma concentration ($C_{\text{max}}$) of about 30% [2–4,6–8,12] to detect 20% differences in the pharmacokinetic parameters between the two formulations, with a power of 80% and an alpha error of 0.05. Subjects were included if they met all the following inclusion criteria: 18–35 years of age, non-smoking, body mass index between 19 and 30, results within the normal range of laboratory values for haematology, clinical chemistry, and urinalysis, negative test results for serum hepatitis B surface antigen, hepatitis C antibody and human immunodeficiency (HIV) antibody, and capable of understanding the requirements of the study. Subjects with known drug hypersensitivity, history of clinically significant psychiatric or medical disease, or history of drug or alcohol abuse, were excluded. Prescription drug treatment was not allowed during the 30 days before study drug administration. Subjects were free to withdraw from the study at any time.

2.2. Study design and procedures

The design was a single-dose, randomised, two-period, crossover study. It was open for investigators, but blind for the analyst responsible for plasma amlodipine concentration measurement. Every subject received a single 10-mg oral dose of each amlodipine formulation separated by a 14-day washout period. The sequence of administration was determined by a randomisation schedule in a balanced manner (blocks of six subjects). Subjects fasted for 10 h prior to and 5 h following drug administration at approximately 9:00; water intake was not allowed in this interval. Subjects were confined to the clinical trial unit for the first 12 h after dosing. Subjects received standard meals during both treatment periods. Meals were served at 5 and 9 h after dosing. Consumption of alcohol- or caffeine-containing beverages and grapefruit juice was prohibited from 48 h prior to dosing until the last blood sampling in each period. During the study period, no medication was allowed unless required to treat any adverse event. Blood samples were collected in 10-mL sterile heparinised tubes pre-dose and at the following times after dosing: 1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 24, 48, 72, 96, 120, 144 and 168 h. An angiocatheter with normal saline lock was inserted into a vein in the antecubital area and was used for sampling during the first 12 h, after discarding 1 mL of blood. Samples were centrifuged for 10 min at 3500 rpm and plasma was stored at −30 °C until analysis. Blood pressure and heart rate were measured using an automatic monitor in supine position at 3, 6 and 9 h and an ECG was done at 8 h post-dose. The number of pharmacodynamic measurements was limited due to the fact that pharmacodynamic analysis was not the main objective of the study; thus, measurements were performed around $T_{\text{max}}$, when maximum effect was expected. QTc, QRS and PR intervals on ECG were automatically calculated by the ECG device.

2.3. Drugs

The following 10-mg amlodipine besylate tablet formulations were employed: Grupo Tecnimede’s amlodipine formulation manufactured by Grupo Tecnimede, Portugal (batch 20647, expiration date 11/2004) as test formulation, and Norvas$^\text{®}$ marketed by Pfizer, Spain (batch S-38, expiration date 09/2006) as reference formulation. Drugs were administered as a single dose by oral route with 200 mL of water. Subjects were under fasting conditions from 10 h before dosing and until 5 h after dose administration.

2.4. Drug assay

The analytical method for amlodipine quantification was developed and validated at SFBC Anapharm (Canada). Drug levels were determined by high-performance liquid chromatographic method with tandem mass spectrometry detection.
Following collection, blood samples were placed in lithium heparinised tubes, and after centrifuging for 10 min at 3000 rpm/4 °C the plasma was separated into two properly identified plasma aliquots. These tubes were immediately frozen at −20 °C and maintained at that or lower temperature. For sample analysis, human plasma was thawed at room temperature and samples were processed after the addition of the internal standard (nalbuphine hydrochloride dehydrate, Sigma, USA). A calibration curve was analysed in the 50.00–7500.00 pg mL⁻¹ range. Samples were extracted with 2:1 tert-butyl-methyl ether (MTBE), and injected into the chromatographic system composed of an automatic sampler (Hitachi L-7250, Chromabec, Canada), and an IB-SIL 5 CN-BD analytical column (Phenomenex, USA). Mass detection was accomplished with API-3000 (MDS Sciex, Canada) and TurbolonSpray (MDS Sciex, Canada).

The validation scheme involved the analysis of calibration curves and quality controls at different concentrations to determine linearity, intra- and inter-assay precision and accuracy, limit of quantification, dilution factor, selectivity, stability and recovery. The analytical phase was performed in Good Laboratory Practice (GLP) environment.

According to the validation results, the method was shown to be linear (r ≥ 0.9962), with an established lower limit of quantification (LLOQ) of 50.00 pg mL⁻¹ with a signal to noise ratio of 14. Between-run accuracy was set at 99.61–104.49% of the quality control’s nominal concentration, while within-run accuracy was 90.98–108.53%. Between- and within-run precisions (in terms of coefficient of variation) were 6.56–7.96 and 2.75–9.58%, respectively. No significant interferences were observed in 10 tested matrices for amlodipine and no effect on analyte quantification was achieved with API-3000 (MDS Sciex, Canada) and TurbolonSpray (MDS Sciex, Canada).

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2.5. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by noncompartmental methods. All calculations were carried out using WinNonlin 2.0 (Scientific Consulting, Inc., USA). The area under the curve for the time of administration to the last measured concentration (AUC₀–∞) was calculated by trapezoidal integration. The total area under the curve from administration to infinity (AUC₀–∞) was calculated as the sum of AUC₀–t and residual area (C₀) divided by kₑ, with C₀ 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. Half-life (t½) was calculated by dividing 0.693 by the kₑ. The peak plasma concentration (Cₘₐₓ) and the time to attain peak (Tₘₐₓ) were obtained directly from the raw data. The total drug clearance adjusted for bioavailability (Cl/F) was calculated by dividing the dose by the AUC. The apparent volume of distribution adjusted for bioavailability (Vd/F) was calculated by dividing the Cl/F by kₑ.

2.6. Statistical analysis

According to international guidelines, the main variables for testing bioequivalence of two formulations are AUC and Cₘₐₓ calculated from the plasma concentrations of amlodipine. Tₘₐₓ has been considered a secondary variable [9–11]. Log-transformed AUC was submitted to analysis of variance (ANOVA) using sequence, subject nested within sequence, period, and treatment as model effects. Treatment sequence was tested against subject within treatment sequence to investigate any carry-over effects. Point estimates and associated 90% confidence interval were obtained in the log-scale for the difference ‘test minus reference preparation’ using the residual variance. Point estimates and associated 90% confidence intervals obtained from the log-scale were then back-transformed to estimate the ‘test/reference’ ratios. Bioequivalence was accepted in case the 90% confidence intervals fell within the pre-defined limits of 80–125% [9–10]. Cₘₐₓ was assessed in a similar fashion.

As Tₘₐₓ is a discontinuous measure, confidence interval was calculated by a non-parametric approach [13–14] and formulations were considered bioequivalent if the 90% confidence interval fell within the limits of 80–120%.

Pharmacokinetic sex differences were evaluated by ANOVA with effects for sex, sequence, interaction sex by sequence, volunteer nested within interaction sex by sequence. Bioequivalence was also evaluated within each gender group as before.

Comparisons of demographic and kinetic parameters between men and women were performed by analysis of variance (ANOVA). Pharmacodynamic parameters were analysed by ANOVA with replication (drug and sex as intersubject factors) followed by Bonferroni multiple comparisons with overall confidence of 95% where appropriate. Chi-square tests or Mann-Whitney tests were used to compare the incidence of adverse reactions.

Results throughout are expressed as mean ± standard deviation of mean (S.D.) Pharmacokinetic and bioequivalence analyses were carried out using WinNonlin 2.0. Other statistical analyses were performed using SPSS version 11.5.1 (SPSS Inc.).

3. Results

Thirty-six Caucasian subjects (18 females and 18 males) were included in this study. All subjects completed the study and were included in the pharmacokinetic and tolerability analysis. The male group was composed of 18 volunteers with a mean ± S.D. age of 23.8 ± 2.6 years, mean ± S.D. height of 176.7 ± 6.2 cm, and weight 73.9 ± 8.0 kg. The female group was also composed by 18 volunteers, with a mean ± S.D. age of 22.7 ± 1.4 years, mean ± S.D. height of 163.4 ± 6.0 cm,
Table 1
Pharmacokinetic parameters for each formulation of amlodipine after a single 10-mg oral dose of two formulations of amlodipine tablets, obtained by a noncompartmental model (n = 36)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tecnimede Norvas®</th>
<th>Norvas®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>AUC∞ (h ng mL⁻¹)</td>
<td>243.3</td>
<td>59.1</td>
</tr>
<tr>
<td>AUCt (h ng mL⁻¹)</td>
<td>232.1</td>
<td>55.6</td>
</tr>
<tr>
<td>AUC% extrapolated</td>
<td>4.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Cl (mg mL⁻¹)</td>
<td>5.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>43.5</td>
<td>9.59</td>
</tr>
<tr>
<td>T₁/₂ (h)</td>
<td>36.8</td>
<td>6.6</td>
</tr>
<tr>
<td>C max (h)</td>
<td>2277.0</td>
<td>567.1</td>
</tr>
<tr>
<td>T max (h)</td>
<td>6.9</td>
<td>2.2</td>
</tr>
<tr>
<td>T max (h) median (range)</td>
<td>6.5 (3–12)</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05.

and weight 59.5 ± 5.6 kg. Nine males and nine females were allocated to each treatment sequence.

3.1. Pharmacokinetic analysis

Table 1 summarises mean pharmacokinetic parameters for both formulations. All pharmacokinetic parameters derived from the model-free approach were similar for test and reference formulations. Almost identical plasma amlodipine concentration profiles were obtained from the two formulations (Fig. 1) showing peak levels around 6 h after drug administration. All subjects showed plasma concentrations above the limit of quantification (50 pg mL⁻¹) 168 h after dosing. Residual AUC was on average 4.5%, ranging between 1.1 and 11.3%. On average, terminal half-life was estimated to be around 37 h for both products. The arithmetic means of the AUC show differences of only 4.6% in terms of the extent of absorption of the formulations, but the ANOVA showed this difference was statistically significant. There was no period or sequence effect. The intersubject variability appeared similar for both formulations (Table 1). Intrasubject coefficients of variation (derived from the mean square error of the ANOVA) were 7.5% (AUC) and 7.4% (Cmax).

Bioequivalence between both formulations was demonstrated with respect to the primary and secondary endpoints (AUC, Cmax and Tmax) since the 90% confidence intervals for the corresponding mean ratios (test/reference) were completely contained in the predefined bioequivalence acceptance range (Table 2).

3.2. Pharmacodynamics and adverse reactions

The figures of blood pressure, heart rate and ECG stayed under physiologic limits during the whole study. There was a significant slight decrease of systolic blood pressure (mean 4 mmHg) at 3 h post-dose for either formulation; however, the diastolic blood pressure did not significantly change at

Fig. 1. Time–concentration profiles of the population means after the administration of a single 10-mg oral dose of reference (circles) and test (diamonds) formulations of amlodipine tablets (n = 36).
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Table 3

Effects of amlodipine on systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR) and electrocardiogram intervals PR, QRS and QTc.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Pre-dose</th>
<th>Post-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>Female</td>
<td>98.2 ± 8.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>113.4 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>108.2 ± 11.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>Female</td>
<td>55.9 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>58.6 ± 7.3</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>57.3 ± 7.1</td>
</tr>
<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Female</td>
<td>70.5 ± 10.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>62.3 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>66.4 ± 10.5</td>
</tr>
<tr>
<td>PR (ms)</td>
<td>Female</td>
<td>155.4 ± 21.6</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>161.4 ± 21.9</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>158.4 ± 21.9</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>Female</td>
<td>89.7 ± 6.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>98.1 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>93.9 ± 9.9</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td>Female</td>
<td>410.4 ± 21.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>398.6 ± 21.7</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>404.5 ± 22.2</td>
</tr>
</tbody>
</table>

All parameters were recorded in supine position in the 36 subjects (18 males and 18 females). Data of both formulations are included. Mean ± S.D.

<sup>a</sup> p < 0.05 compared with pre-dose value.

<sup>b</sup> p < 0.05 compared with male value.

<sup>8</sup> h post-dose.

any time (mean reduction of 1.9 mmHg at 6 h post-dose) (Table 3). At 6 and 9 h post-dose, there was an increase in heart rate (mean of 9.9 beats min<sup>-1</sup> with the test formulation and 8.3 beats min<sup>-1</sup> with the reference drug at 6 h). The electrocardiogram showed a slight decrease of PR and QRS intervals at 8 h post-dose that could be related to the increase in heart rate. The QTc interval did not change. There were no significant differences between formulations on pharmacodynamic parameters (Fig. 2).

During the study, 41 adverse events, which were considered probable or possibly related to treatment (adverse reactions) were reported. Twenty subjects (55.6%) reported 27 adverse reactions after exposure to the test formulation as compared to 14 adverse reactions in 11 subjects (30.6%) after exposure to the reference formulation. No severe, serious or life threatening adverse events were reported during the course of the study. No adverse event caused premature termination of the study. Except for four episodes of moderate headache, all adverse reactions were of mild intensity. All events were resolved at the end of the trial. The most common adverse reactions were headache, dizziness and tiredness that were reported by 33, 17 and 8% of subjects after receiving the test formulation, respectively, and 19, 6 and 6% after the reference formulation (non-significant difference).

No analytical significant alterations, which could be attributed to treatment, were detected. A mean decrease of 0.7 g/dL<sup>-1</sup> was observed for haemoglobin, which is most likely related to the collection of blood samples during the study.

3.3. Gender differences

On average, bioavailability of amlodipine was slightly higher in females than in males (Fig. 3). However, these differences in pharmacokinetic parameters could be attributed to the lower body weight of women, because the differences became non-significant when data were adjusted for weight (Table 4). Bioequivalence was also demonstrated within each gender group. For males, the mean AUC ratio (test/reference) was estimated as 104.5% with a 90% confidence interval of 98.8–110.6, and the mean C<sub>max</sub> ratio as 99.4% with a...
Fig. 3. Mean plasma concentrations vs. time curve for males (continuous line) and females (discontinuous line) after the administration of a single 10-mg oral dose of amlodipine tablets. Means of test and reference formulation (n = 18 for each group).

90% confidence interval of 94.4–105.0. Similar results were obtained for females, the mean AUC ratio (test/reference) was estimated as 104.6% with a 90% confidence interval of 101.6–107.1, and the mean C_max ratio as 104.6% with a 90% confidence interval of 101.6–107.1, and the mean C_max ratio as 101.3 with a 90% confidence interval of 97.7–105.0.

There were significant differences between males and females in systolic and diastolic blood pressure, heart rate, and QRS and QTc intervals of the electrocardiogram (Table 3). There was a higher increase in heart rate at 6 h post-dose in females (13.5 beats min⁻¹) than in males (4.6 beats min⁻¹). The incidence of adverse reactions was similar in men (56% reported at least one adverse reaction with any formulation) and in women (61%). Headache, dizziness and tiredness were reported by 44, 11 and 17% of men, and 28, 28 and 6% of women, respectively (non-significant difference).

4. Discussion

In this study of 36 healthy subjects, it was shown that the two 10-mg amlodipine formulations are bioequivalent according to the recommendations of the European and US regulatory frameworks on the investigation of bioavailability and bioequivalence [9–11]. The 90% confidence intervals for the primary endpoint AUC and C_max were completely contained within the pre-defined bioequivalence acceptance range of 80–125%. Bioequivalence was also demonstrated for T_max with 90% confidence interval within the limit 80–120%.

All pharmacokinetic parameters derived from the non-compartmental approach showed very similar results for both formulations. They are also similar to those already published in the literature [1–8]. Although there was a significant difference between the two formulations in AUC (see Table 1), this difference is not clinically significant because it is smaller than 8%, as can be seen from the 90% confidence interval of 101.5–107.7%.

On average, terminal half-life was estimated to be around 37 h for both products, which is consistent with published results for amlodipine [1–8,12,16]. The individual half-lives ranged between 24 and 55 h, so the 14-day washout period was enough because it is longer than six half-lives for all the subjects. In fact, no carry-over or period effects were indicated by statistical analyses.

Table 4

Pharmacokinetic parameters of amlodipine for males and females after a single 10-mg oral dose of two formulations of amlodipine tablets, obtained by a noncompartmental model (n = 18 for each group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose–weight (mg kg⁻¹)</td>
<td>0.137 ± 0.013</td>
<td>0.170 ± 0.016</td>
<td>0.0001</td>
</tr>
<tr>
<td>AUC (ng mL⁻¹ · h⁻¹)</td>
<td>217.4 ± 43.4</td>
<td>258.8 ± 63.1</td>
<td>0.0233</td>
</tr>
<tr>
<td>AUC₀–∞ (ng mL⁻¹)</td>
<td>264.5 ± 281.1</td>
<td>1536.9 ± 362.3</td>
<td>0.8903</td>
</tr>
<tr>
<td>C_max (mg mL⁻¹)</td>
<td>4.6 ± 0.9</td>
<td>5.7 ± 1.1</td>
<td>0.0009</td>
</tr>
<tr>
<td>C_max (mg mL⁻¹ · dose–weight)</td>
<td>33.5 ± 5.2</td>
<td>33.6 ± 6.3</td>
<td>0.4210</td>
</tr>
<tr>
<td>T_max (h) mean</td>
<td>6.6 ± 1.8</td>
<td>7.1 ± 2.1</td>
<td>0.4695</td>
</tr>
<tr>
<td>T_max (h) median (range)</td>
<td>6.0 (3–12)</td>
<td>6.5 (3–12)</td>
<td></td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>39.4 ± 5.5</td>
<td>34.6 ± 3.4</td>
<td>0.0062</td>
</tr>
<tr>
<td>Vd/F (L)</td>
<td>2678.5 ± 457.0</td>
<td>1995.5 ± 393.7</td>
<td>0.0001</td>
</tr>
<tr>
<td>Vd/F (L · kg⁻¹)</td>
<td>36.4 ± 6.1</td>
<td>33.6 ± 6.3</td>
<td>0.1358</td>
</tr>
<tr>
<td>CVF (L · h⁻¹)</td>
<td>47.6 ± 8.3</td>
<td>41.0 ± 10.3</td>
<td>0.0233</td>
</tr>
<tr>
<td>CVF (L · h⁻¹ · kg⁻¹)</td>
<td>0.65 ± 0.11</td>
<td>0.69 ± 0.18</td>
<td>0.4210</td>
</tr>
</tbody>
</table>

Data of both formulations are included.
Intrasubject CV% was much lower than the anticipated 30%. As they are smaller than the published data [2–8,12,16], a smaller sample size would have been enough to show bioequivalence. Indeed, bioequivalence was shown for each gender with only 18 subjects per group and three papers in which 18–24 subjects were included to show bioequivalence have been previously published [16–18]. This means that the power of the study even markedly exceeds the anticipated power of 90% based on 36 subjects, and that a high credibility of the study results is provided. The study has therefore enough power for comparisons between males and females with 18 subjects per group. Furthermore, the low intrasubject CV has also contributed to detect a small significant difference in AUC, since larger sample sizes allow for the detection of small differences. However, statistical difference is clinically irrelevant since the 90% confidence intervals are well within the predefined ranges.

The study was balanced with regard to gender, within the randomised sequence groups, which means: nine males and nine females were allocated to the treatment sequence “test-reference”, and nine males and nine females were allocated to the treatment sequence “reference-test”. Accordingly, the possible differences between males and females did not restrict the validity of the bioequivalence assessment based on intrasubject comparisons. Some isoenzymes of the cytochrome P450 CYP2 and CYP3 families are sex-specific or are regulated by sex hormones [19–21]. For drugs that are metabolised by conjugation or oxidation, clearance is slower in women [20–21]. For other drugs, cleared by hydroxylation and demethylation reactions, such as diazepam, prednisolone, theophylline and erythromycin, clearance is greater in women [20–22]. As amlodipine is metabolised by CYP3A4 [6], the same as β-estradiol [23], it is possible that clearance of amlodipine could be different in women. A higher bioavailability has been found for females than for males, however this difference may be explained by the higher dose per weight unit administered to women. Indeed, there are no significant differences in clearance adjusted by weight. However, there are some clinical trials where women had a greater blood pressure response to amlodipine [24], which can be related to these higher amlodipine concentrations. Women usually present higher intrasubject variability in pharmacokinetic parameters than men [15], but this is not the case with amlodipine since variability seems smaller in women (see narrower bioequivalence intervals in women).

Other factors that could influence the pharmacokinetics of amlodipine were avoided: none of the volunteers smoked, they did not receive other drugs, age was similar in men and women, administration time and food intake were the same every day. One of the main objections to the studies evaluating sex differences is that investigators do not consider what part of the menstrual cycle the women were and whether they were taking oral contraceptives [22,25]. In this study, no women were receiving contraceptives, but the phase of the menstrual cycle was not considered upon enrolment. As the washout period was 14 days, 16 of the women received each one of the formulations in different phases of the menstrual cycle and no differences were observed in clearance or other parameters, suggesting that menstrual cycle may not play a relevant role in amlodipine pharmacokinetics. However, a new study with a different design would be necessary to evaluate this hypothesis.

Therefore, the data obtained in this study show no relevant sex differences in the pharmacokinetics of amlodipine. In any case, if there were any differences undetectable by this study, they would probably be of minor clinical relevance. It is also noteworthy to emphasise that bioequivalence trials provide very relevant data in terms of evaluation of pharmacokinetic (i.e. sex differences) or pharmacodynamic characteristics of drugs, getting a higher benefit from investigation with healthy volunteers without damaging the main endpoint of the study.

Based on the mechanism of action established for amlodipine, the expectable changes in vital signs were noted with both treatments. As it is known, the decrease in blood pressure is smaller than that observed in hypertensive patients, as other single dose studies have shown in healthy volunteers [3,5–6]. However, this study does not include a placebo group and it is not possible to ascertain whether changes in blood pressure and heart rate are due to circadian fluctuations, as has been shown in other single dose studies [7,12]. Nevertheless, a recent study shows a higher reduction in blood pressure with a single 5-mg amlodipine dose [17], which could be related to pharmacokinetic/pharmacodynamic questions related to ethnic factors (Korean subjects) or to the use of different blood pressure measurement techniques (blood pressure measured in a sitting position). On the other hand, it has also been shown that blood pressure is lower in women than in men, and that women usually present a higher heart rate and QTc interval in the electrocardiogram.

Analysis of the tolerability data revealed no evidence of a treatment difference in the incidence, severity, or causality of adverse events. All adverse events were mild or moderate in intensity, and no serious adverse events were reported. Both the incidence and intensity of adverse events reported during the study period are consistent with the amlodipine product label, and did not suggest a treatment difference [1]. Headache was the most common adverse reaction, as in other healthy volunteers studies [16]. Although amlodipine concentrations were higher in women, they did not show a higher incidence of adverse reactions.

In conclusion, the two formulations were bioequivalent in terms of the rate and extent of absorption, according to the prescribed bioequivalence criteria of the regulatory bodies. Moreover, they were bioequivalent within each gender group. Women reached higher amlodipine concentrations because of their lower body weight, and consequently they exhibited greater pharmacodynamic effects. However, there were no sex differences in drug clearance.
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References