COMPARATIVE BIOAVAILABILITY OF A FIXED-DOSE COMBINATION TABLET OF OLMESARTAN MEDOXOMIL / HYDROCHLOROTHIAZIDE IN HEALTHY KOREAN VOLUNTEERS

RENHUA ZHENG¹, HO MIN HWANG¹ and BO-HYUNG KIM¹ ² ³

¹Department of Medicine, Graduate School, Kyung Hee University, Seoul, Republic of Korea
²Department of Clinical Pharmacology and Therapeutics; ³East-West Medical Research Institute, Kyung Hee University College of Medicine and Hospital, 26 Kyunghheedae-ro, Dongdaemun-gu, Seoul 02447, Republic of Korea

Abstract: Combination therapy with diuretics and angiotensin II type 1 (AT1) receptor antagonist is frequently recommended for the control of blood pressure in hypertensive patients. This study was targeted to compare pharmacokinetic profiles of a new generic fixed-dose combination (FDC) tablet of olmesartan medoxomil/hydrochlorothiazide 20/12.5 mg and a reference formulation of Olmetec Plus® 20/12.5 mg tablets in healthy volunteers. The study design was a randomized sequence and two-way crossover study in healthy subjects. They were to be randomly assigned to either one of the two sequence groups; each subject sequentially received a single oral dose of reference and test tablet with 7-day washout period. Blood sample was collected at pre-dose and at 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 h post-dose. The blood concentrations were analyzed by LC-MS/MS. Both of the 90% CI for the treatment ratios (test/reference) of Cmax and AUClast were to be in the range of 0.800-1.250 with regards to olmesartan medoxomil and hydrochlorothiazide; the geometric mean ratios (test/reference) for olmesartan Cmax and AUClast were 0.979 (90% CI, 0.934-1.027) and 0.992 (0.946-1.041), respectively, and those for hydrochlorothiazide Cmax and AUClast were 0.966 (0.973-1.110) and 0.999 (0.963-1.038), respectively. No serious adverse events were reported during the study. The generic formulation of olmesartan medoxomil/hydrochlorothiazide 20/12.5 mg tablet was bioequivalent with the reference formulation of Olmetec Plus® 20/12.5 mg tablet in regards to the pharmacokinetic parameters of olmesartan medoxomil and hydrochlorothiazide. Clinical Research Information Service (CRIS) Registration Number: KCT0001025. (https://cris.nih.go.kr/ Mar 18, 2014)

Keywords: olmesartan medoxomil; hydrochlorothiazide; combination tablet; pharmacokinetics; bioequivalence

Hypertension is one of the powerful risk factors for cardiovascular disease including myocardial infarction, stroke, and heart failure (1, 2). To decrease cardiovascular disease incidence, the control of blood pressure (BP) in hypertensive patients is critical. However, the control rate (≤ 140/90 mmHg) of BP for those who take antihypertensive drugs was 50.2, 54.0 and 56.6% at 1999-2000, 2001-2002, and 2003-2004, respectively, according to the analysis of database from the National Health and Nutrition Examination Survey (3). Therefore, a combination therapy with complementary mechanisms is often required to control BP in clinical practice (4, 5).

Thiazide is an effective diuretic for the control of BP by augmenting the excretion of water and sodium (6). However, these volume contractions increase the activity of renin angiotensin system throughout the stimulation of renin release. Angiotensin II type 1 (AT₁) receptor antagonists inhibit the increment of BP by angiotensin II (7). Therefore, combination treatment of thiazide with AT₁ receptor blocker is more effective than monotherapy with either thiazide or AT₁ receptor blocker (8). This synergistic effect was demonstrated through several clinical trials, in which olmesartan medoxomil, as one of AT₁ receptor blockers with hydrochlorothiazide (HCTZ), reduced diastolic and systolic BP to a greater extent than monotherapy with either component (9, 10). In addition, initial combination therapy was significantly associated with the reduced risk of cardiovascular events in

* Corresponding author: e-mail: bhkim98@khu.ac.kr; phone: (office) +82-2-958-9326; fax: +82-2-958-9559
Figure 1. Mean (SD) plasma concentration-time curve of olmesartan in linear scale (A) and log-linear scale (B) after the oral administration of olmesartan medoxomil/HCTZ FDC tablet to healthy volunteers.
Figure 2. Mean (SD) plasma concentration-time curve of HCTZ in linear scale (A) and log-linear scale (B) after the oral administration of olmesartan medoxomil/HCTZ FDC tablet to healthy volunteers.
hypertensive patients, compared with matched patients who initially received an anti-hypertensive agent as monotherapy with the subsequent administration of another agent (11).

Meanwhile, a meta-analysis for medication compliance reported that the risk of non-compliance for patients administered FDC decreased, compared to free-drug (12). Therefore, a Korean domestic company developed a new generic formulation of olmesartan medoxomil/HCTZ tablets. This study was conducted to compare the pharmacokinetic profiles of a new generic formulation of olmesartan medoxomil/HCTZ tablets to those of branded reference formulation.

EXPERIMENTAL

Subjects and Methods

Participants

A total of forty four healthy volunteers (aged from 19 to 55 years) with normal height and weight were enrolled in the study. The body weights were within ± 20% of their ideal body weight (IBW) which was calculated using the equation: [height (cm) – 100] × 0.9. Subjects were screened by physical examination, clinical laboratory test, 12-lead electrocardiography and medication history. All subjects had provided written informed consent prior to the study and the study was conducted in accordance with the guideline for good clinical practice of International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (version 4, June, 1996, Efficacy Guidelines, ICH-E6). The study protocol was reviewed and approved by the institutional review board at the Kyung Hee University Hospital.

Study design

This clinical trial was a randomized sequence, open-label, single-dose, two-way crossover design to evaluate the pharmacokinetics of olmesartan and HCTZ for test and reference formulations.

All subjects were assigned to either one of two sequence groups (22 subjects in each group). One sequence group received a single dose of reference tablet while the other group received a single dose of test tablet. Then, all subjects received an opposite treatment with a washout period of 1 week. All subjects were hospitalized at the clinical trials center at the Kyung Hee University Hospital at 6 p.m. on the day before dosing. On the next day, they received a test or reference drug with 240 mL tap of water; they were then discharged 48 h after the dose. They were readmitted to the same center after 1 week, received an opposite treatment, and were then discharged 48 h after the 2nd dose. Meals were given to all subjects at 4 and 10 h after the drug administration.

Pharmacokinetic sampling and analysis

The blood samples of 7 mL were sequentially collected at the following scheduled time-points; pre-dose and 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 6, 8, 12, 24, 36 and 48 h after the oral administration of study formulations. The blood samples were collected into heparinized tubes, which were centrifuged at 3000 rpm for 10 min. The supernatant plasma was transferred into two Eppendorf tubes and then stored at -70°C until determination of drug concentration.

The plasma concentrations of olmesartan were determined using an isocratic high-performance liquid chromatography (HPLC, Agilent 1200 Series, Agilent Technologies, Waldbronn Germany) equipped with a Gemini® C18 column (3 µm, 50 × 2.0 mm; Phenomenex, Torrance, USA). The HPLC was coupled with a 6460 triple quadrupole mass spectrometer (MS/MS, Agilent Technologies, Waldbronn, Germany). Quantification was performed in the positive ion multiple reaction monitoring (MRM) mode; m/z = 447.6 → 206.8 for olmesartan, m/z = 423.1 → m/z 206.8 for losartan (internal standard, IS) (13). An aliquot of 100 µL human plasma was added with 600 µL of IS solution (10 ng/mL) which was prepared with acetonitrile (extract solution). The mixture was vortexed for 2 min and centrifuged for 10 min (13000 rpm, 4°C), and then 1 µL aliquot of supernatant was injected into the LC-MS/MS system. The mobile phase was a mixture of formic acid/acetonitrile/1 mM ammonium acetate (0.1 : 50 : 50, v/v/v) at a flow rate of 0.35 mL/min. The calibration curve of olmesartan was linear from 5 ng/mL to 1000 ng/mL (r² ≥ 0.99). The precision and accuracy tests were conducted for lower limit of quantification (LLOQ) and three quality control (QC) samples (low QC, 10 ng/mL; middle QC, 500 ng/mL; high QC, 800 ng/mL). The intra- and inter-day precisions were 2.05-8.32% and 3.90-7.37%, respectively. The accuracy of intra-day and inter-day ranged from 91.1-97.4% and 89.8-97.4% of nominal value, respectively.

The plasma concentrations of HCTZ were determined using an isocratic HPLC (Agilent 1200 Series, Agilent Technologies, Waldbronn, Germany) equipped with a Gemini® C18 column (3 µm, 50 × 2.0 mm; Phenomenex, Torrance, USA). The HPLC system was coupled with a 6410 triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). Quantification was
performed in negative ion MRM mode; m/z = 296.1 → 204.9 for HCTZ, m/z = 299.2 → m/z 205.8 for HCTZ-D2 (IS) (14). The IS solution was prepared at 2 µg/mL using acetonitrile. An aliquot of 100 µL human plasma was mixed with 50 µL of IS solution and 4 mL of methyl tert-butyl ether (MTBE) solution, and then the mixtures were vortexed for 20 min. After centrifuging for 5 min (4000 rpm, 4°C), 3 mL aliquot of supernatant was transferred into the other tube and evaporated under nitrogen gas (45°C). The residue was reconstituted in the mobile phase, and then 3 µL aliquot of this solution was injected into the LC-MS/MS system. The mobile phase was a mixture of water/acetonitrile (10 : 90, v/v) at a flow rate of 0.3 mL/min. The calibration curve of HCTZ was linear from 1 ng/mL to 200 ng/mL ($r^2$ ≥ 0.99). The precision and accuracy tests were conducted for LLOQ and three QC samples (low QC, 2.5 ng/mL; middle QC, 100 ng/mL; high QC, 160 ng/mL). The intra- and inter-day precisions were 1.79-6.76% and 2.55-7.20%, respectively. The accuracy of intra-day and inter-day ranged from 94.9 to 107.5% and from 102.7 to 106.1%, respectively.

Both olmesartan and HCTZ in plasma samples were stable at room temperature for 24 h and were unaffected by 3 freeze-thaw cycles (< ±15% versus the fresh sample). All the procedures including method validation followed the Korean Food and Drug Administration (FDA) and US FDA guidelines (15, 16).

**Tolerability assessments**

The tolerability of study medicines was evaluated as follows; clinical laboratory tests, electrocardiographies and vital signs were assessed before and after dosing of study medicines; the medical history was recorded by the study staff, and adverse events (AEs) were investigated during the study; the laboratory results were obtained by the qualified staff at the Department of Diagnostic Laboratory Medicine at Kyung Hee University Hospital. The above assessments were performed at scheduled time points throughout the study.

**Pharmacokinetic evaluation**

The pharmacokinetic parameters were calculated using a non-compartmental method, using Phoenix® WinNonlin® 6.2 (Pharsight, a Certara™ Company, St. Louis, MO, USA). The maximum plasma concentrations (Cmax) and the time to reach the peak concentration (tmax) were observed from the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test</th>
<th>Reference</th>
<th>ANOVA F-value (p-value)</th>
<th>GMR (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olmesartan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2 (1 - 4)</td>
<td>1.67 (1 - 4)</td>
<td>-</td>
<td>0.412*</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>665.9 ± 150.6 [650.84]</td>
<td>681.1 ± 157.3 [664.14]</td>
<td>0.54 (0.468)</td>
<td>0.979 (0.934-1.027)</td>
</tr>
<tr>
<td>AUClast (ng◊h/mL)</td>
<td>4244.2 ± 958.8 [4145.35]</td>
<td>4252.2 ± 831.9 [4174.36]</td>
<td>0.07 (0.788)</td>
<td>0.992 (0.946-1.041)</td>
</tr>
<tr>
<td>AUCinf (ng◊h/mL)</td>
<td>4348.8 ± 959.1 [4252.34]</td>
<td>4357.6 ± 846.6 [4278.43]</td>
<td>0.38 (0.539)</td>
<td>0.995 (0.950-1.043)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>8.60 ± 2.52</td>
<td>8.78 ± 3.21</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCTZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.84 (1 - 4)</td>
<td>1.67 (1 - 4)</td>
<td>-</td>
<td>0.640*</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>76.11 ± 22.47 [72.69]</td>
<td>78.69 ± 23.98 [75.39]</td>
<td>0.76 (0.388)</td>
<td>0.966 (0.975-1.110)</td>
</tr>
<tr>
<td>AUClast (ng◊h/mL)</td>
<td>453.8 ± 86.5 [445.92]</td>
<td>455.0 ± 94.3 [446.17]</td>
<td>&lt; 0.01 (0.974)</td>
<td>0.999 (0.963-1.038)</td>
</tr>
<tr>
<td>AUCinf (ng◊h/mL)</td>
<td>476.0 ± 88.73 [469.48]</td>
<td>477.8 ± 95.90 [470.06]</td>
<td>&lt; 0.01 (0.947)</td>
<td>0.999 (0.964-1.035)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>9.43 ± 1.88</td>
<td>9.28 ± 1.40</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All values are represented as arithmetic mean ± standard deviation with [geometric mean]. AUClast = area under the time-concentration curve from time zero to time of the last measurable concentration; AUCinf = AUC from time zero to infinity; Cmax, maximum plasma concentration; t1/2, terminal elimination half-life; tmax, time to reach Cmax; * GMR, geometric mean ratio (90% CI) of test to reference formulations; 'Tmax is shown as median (minimum-maximum); ' p-value using the Wilcoxon signed-rank test.
individual plasma concentration curves. The dosing to the time of the last quantifiable concentration (AUC_{last}) was calculated by the linear trapezoidal rule, and AUC from dosing to time infinity (AUC_{inf}) was obtained from summation of AUC_{last} and C_{last}/λ_z. The individual terminal elimination rate constant (λ_z) was calculated by the linear regression of the log-linear decline of plasma concentration versus time data in the terminal phase. The terminal elimination half-life (t_{1/2}) was determined as 0.693/λ_z.

Data analysis and statistics
A total of 40 subjects data were calculated at 5% significant level with 90% CI based on a previously reported equation (17). This sample size was obtained based on the following assumptions; the geometric mean pharmacokinetic parameter treatment ratio (test/reference) was 1 and intra-subject CV for pharmacokinetic parameters was 30%. Four subjects were additionally needed due to withdrawal from the study.

The SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The logarithm-transferred C_{max} and AUC_{last} were compared between the test and the reference formulations, using a generalized linear mixed model with the fixed effects of “sequence”, “period” and “treatment”, and the random effect of “subjects nested within the sequence”. The mean differences with a 90% confidence interval (CI) between both formulation groups were transformed into anti-log values to calculate the geometric mean ratios and CI of the ratios. The Wilcoxon signed rank test was used to compare T_{max} between the test and reference groups for olmesartan medoxomil and HCTZ.

RESULTS
Demographic characteristics
A total of 44 healthy volunteers participated in the study and were assigned into two sequence groups. However, two subjects were discontinued due to consent withdrawal. Of the remaining 42 subjects, two were discontinued after a single dose of either the reference or test formulation; one was discontinued due to AEs after the administration of a test formulation at period 1, and the other was discontinued due to consent withdrawal just before the administration of a reference formulation in period 2. The ages of all participants (n = 44) were from 20 to 36 years (mean age ± SD: 24.6 ± 3.4 years), and their body weights were from 54.0 to 88.0 kg (70.2 ± 7.6 kg). Their heights were from 164 to 184 cm (175.4 ± 4.43 cm).

Pharmacokinetic profiles
The mean concentration-time profiles of olmesartan were similar between test and reference drugs (Fig. 1). The olmesartan C_{max} was 665.9 ± 150.6 ng/mL (mean ± SD) for the test drug and 681.1 ± 157.3 ng/mL for the reference drug (Table 1). The olmesartan AUC_{last} was 4244.2 ± 958.8 ng◊h/mL for the test and 4252.2 ± 831.9 ng◊h/mL for the reference. The geometric mean ratio of the test group to the reference group was 0.979 (0.934-1.027) for C_{max} and 0.992 (90% CI, 0.946-1.041) for AUC_{last}.

The mean concentration-time profiles of HCTZ were also similar between the two treatment groups. The HCTZ C_{max} was 76.11 ± 22.47 ng/mL for the test drug and 78.69 ± 23.98 ng/mL for the reference drug. The HCTZ AUC_{last} was 453.8 ± 86.5 ng◊h/mL for the test and 455.0 ± 94.3 ng◊h/mL for the reference. The geometric mean ratio (test/reference) was 0.966 (90% CI, 0.975-1.110) for C_{max} and 0.999 (0.963-1.038) for AUC_{last}.

Tolerability
The test and the reference formulations were well tolerated during the study period with no serious AEs. There was no report of any drug related AEs after the oral administration of the test and the reference formulations in 40 subjects who completed the study. Among the 4 withdrawn subjects, three were consent withdrawal while one subject with test formulation reported dizziness and sweating which were considered drug related AEs (< 30min).

DISCUSSION
The present study indicating the pharmacokinetic profiles of olmesartan and HCTZ can be comparable between the test and the reference formulations. Pharmacokinetic analyses demonstrated that the test formulation of olmesartan medoxomil/HCTZ was bioequivalent to the reference formulation; the geometric mean ratio for olmesartan C_{max} was 0.979 (90% CI, 0.934-1.027) and AUC_{last} 0.992 (90% CI, 0.946-1.041) between both formulations; the geometric mean ratio for HCTZ C_{max} was 0.966 (90% CI, 0.975-1.110) and AUC_{last} 0.999 (90% CI, 0.963-1.038) between both formulations. Olmesartan medoxomil/HCTZ tablet of the reference and test were well tolerated in all subjects who completed the study.

Previous pharmacokinetic studies of olmesartan medoxomil 20 mg tablet reported that C_{max} / AUC_{0-24h} were 681 ± 132 mg/mL (mean ± SD, calculated as CV%, 19.38) / 497 ± 210 ng◊h/mL (43.84%) /
2613 ± 675 ng*h/mL (25.83%) for US study (19). Another previous study of a single dose of enalapril/HCTZ 20/12.5 mg reported that $C_{\text{max}} / AUC$ were 72.5 ± 13.8 ng/mL (19.03%) / 418 ± 115 ng*h/mL (27.51%) for HCTZ (20). Meanwhile, in the present study, the CV (%) of olmesartan medoxomil and HCTZ $C_{\text{max}} / AUC_{\text{last}}$ for the test formulation were 22.62/22.59% and 29.52/20.07%, respectively. Therefore, we thought that pharmacokinetic parameter and inter-individual variabilities of olmesartan and HCTZ for the test formulation used in the present study were not significantly different from those reported values in the previous studies.

The F values of treatment effect for olmesartan $C_{\text{max}}$, $AUC_{\text{last}}$ and $AUC_{\text{inf}}$ were 0.54, 0.07 and 0.38, and those for HCTZ $C_{\text{max}}$, $AUC_{\text{last}}$ and $AUC_{\text{inf}}$ were 0.76, < 0.01 and < 0.01. All of the F values were lower than the F critical value of 4.098 ($d_{f1} = 1$, $d_{f2} = 38$); the treatment effect between both formulations of the reference and test tablets was considered to be not significant (Table 1).

The efficacy and safety of olmesartan medoxomil/HCTZ combination therapy has been proved in a randomized double-blind, multicenter study which reported that the combination therapy of olmesartan medoxomil and HCTZ formulations significantly reduced both seated diastolic BP and seated systolic BP more than the monotherapy of either olmesartan medoxomil or HCTZ, and that there was no clinically relevant differences in the incidence of treatment-emergent AE among placebo (57.1%), HCTZ monotherapy (51.1%), olmesartan medoxomil monotherapy (49.6%), and the combination groups (57.1%) (9). Also, the guideline of the 2007 European Society of Hypertension / European Society of Cardiology (ESH-ESC) indicated that combination therapy of antihypertensive agents was more available than monotherapy; combination therapy with low dose in which side effects occur less likely, compared to full dose monotherapy, earlier control of high BP using combination therapy than monotherapy. Furthermore, a single FDC tablet could simplify the treatment regimen and optimize medication compliance (21).

CONCLUSION

The pharmacokinetic parameters for olmesartan and HCTZ are bioequivalent between the test and the reference formulations of olmesartan medoxomil/HCTZ 20/12.5 mg tablet in healthy Korean volunteers. Both formulations were generally well-tolerated during the study period, with no serious AEs.

Acknowledgments

This study was sponsored by Dong Wha Pharmaceutical Co., Ltd Seoul, Republic of Korea. The authors have indicated that they have no other conflict of interests regarding the content of this article.

The authors are grateful to the study participants, clinical investigators, study coordinators, and the clinical research associates who made this study possible.

REFERENCES

16. RENHUA ZHENG et al.  
ancecomplianceregulatoryinformation/guidan-
(2009).
20. Maya M.T., Goncalves N.J., Silva N.E., Filipe 
Pharmacokinet. 27, 91 (2002).
21. Mansia G., De Backer G., Dominiczak A., 
Ciňova R., Fagard R. et al.: Blood Press. 16, 
135 (2007).

Received: 16. 01. 2015