Pharmacokinetics of atorvastatin and sustained-release metformin fixed-dose combination tablets: two randomized, open-label, 2-way crossover studies in healthy male subjects under fed conditions

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Two separate studies were conducted to establish bioequivalence (BE) for two doses of atorvastatin/metformin sustained-release (SR) fixed dose combination (FDC) versus the same dosage of the individual component (IC) tablets in healthy male subjects under fed conditions (study 1, BE of atorvastatin/metformin SR 20/500 mg FDC; study 2, BE of atorvastatin/metformin SR 20/750 mg FDC). Each study was a randomized, open-label, single oral dose, two-way crossover design. Serial blood samples were collected pre-dose and up to 36 hours post-dose for atorvastatin and 24 hours for metformin. Plasma concentrations of atorvastatin, 2-OH atorvastatin and metformin were analyzed using a validated liquid chromatography tandem mass-spectrometry. A non-compartmental analysis was used to calculate pharmacokinetic (PK) variables and analysis of variance was performed on the lognormal-transformed PK variables. A total of 75 subjects completed the study 1 (36 subjects) and study 2 (39 subjects). The 90% confidence intervals for the adjusted geometric mean ratio of Cmax and the AUC0-t were within the predefined 0.80 to 1.25 range. The number of subjects reporting at least one adverse event following FDC treatments was comparable to that following IC treatments. The two treatments were well tolerated. Therefore, atorvastatin/metformin SR 20/500 mg and 20/750 mg FDC tablets are expected to be used as alternatives to IC tablets to decrease the pill burden and increase patient compliance.

Keywords
Pharmacokinetics, Fixed-dose combination, atorvastatin, metformin extended release

Introduction

Patients with type 2 diabetes have an increased prevalence of lipid abnormalities, contributing to their high risk of cardiovascular disease (CVD). For the past decade or more, multiple clinical trials demonstrated significant effects of statins on CVD outcomes in subjects with coronary heart disease (CHD) and for primary CVD prevention.[1]

Low levels of high-density lipoprotein (HDL) cholesterol and elevated triglyceride levels are the most prevalent pattern of dyslipidemia in persons with type 2 diabetes.[2] Although the evidence base for drugs that target these lipid fractions is significantly less robust than that for statin therapy, for most patients with diabetes, the first priority of dyslipidemia therapy is to lower low-density lipoprotein (LDL) cholesterol to a target...
Study 1 was conducted to establish the bioequivalence of atorvastatin and metformin sustained-release (SR) was developed for patients who might drop out or fail to adhere to both treatments. Each study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki, the guidelines on Good Clinical Practice, and locally applicable laws and regulations. The study protocols, amendments, and the informed consent forms were approved by the Institutional Review Boards of the study site (IRB number: 14-062 and 15-0012). All subjects gave written informed consent prior to commencing the study. The studies were conducted at Clinical Trial Center of Busan Paik Hospital, Busan, Republic of Korea (ClinicalTrials.gov Identifier: NCT02185066 and NCT02378441).

Study Design

Both studies were single-center, randomized, open-label, single-oral dose, two-treatment, two-period, crossover clinical trials enrolling healthy male subjects. Study 1 was conducted to establish the bioequivalence of atorvastatin/metformin SR 20 mg/500 mg FDC tablet (CJ-30056 20/500 mg; CJ HealthCare Co., Ltd., Korea) to atorvastatin 20 mg (Lipitor® tablet 20 mg; Pfizer Pharmaceutical Korea Ltd., Seoul, Republic of Korea) and metformin extended release 500 mg (Glucophage® XR tablet 500 mg; Merck Ltd. Korea, Seoul, Republic of Korea) administered concurrently as IC tablets. In Study 2, subjects administered each of atorvastatin/metformin SR 20 mg/750 mg FDC tablet (CJ-30056 20/750 mg; CJ HealthCare Co., Ltd., Korea) or atorvastatin 20 mg (Lipitor®; Pfizer Pharmaceutical Korea Ltd., Seoul, Republic of Korea) and metformin prolonged release 750 mg (Glucophage® SR tablet 750 mg; Merck Ltd., Feltham, UK).

A single oral dose of the FDC or IC treatment was administered under fed conditions during each period of the study along with 240 mL of water at ambient temperature, under the supervision of trained study personnel. Although atorvastatin can be administered with or without food, metformin should be taken with meals. Thus each study was conducted under fed conditions; subjects started the a high-fat meal 30 minutes prior to administration of drug. During both the study periods, allocation of subjects to the FDC and IC treatments took place according to the randomization schedule generated by SAS® software (SAS Institute, Cary, NC) using a random element to reduce the bias. The two treatments were separated by a wash-out period of 7 days. Assuming that intra-individual coefficient of variation of atorvastatin is 31.9% and the true ratio is one, a sample size of 36 subjects was estimated to have at least 80% power to detect bioequivalence based on the criteria of the 90% confidence interval (CI) for the geometric mean ratio (GMR) falling within the acceptance limit of 0.8–1.25.[13] We started each study with 42 subjects, which provided a contingency for patients who might drop out or fail to adhere to both treatments.

Subjects

Forty-two healthy male subjects aged 19–55 years were enrolled in each study. Subjects were eligible to participate if their weight was within 20% of ideal body weight in accordance with Broca’s formula. Subjects were considered healthy based on their medical history, physical examinations, 12-lead electrocardiography, and clinical laboratory tests. Subjects with a medical history or diet that might interfere with drug absorption, distribution, metabolism, or excretion were excluded. Subjects were also excluded if any of the following applied: history of allergy or hypersensitivity to investigational products, history of drug and/or alcohol abuse, participation in a clinical trial within 60 days of first administration of the investigational product, donation of blood within 60 days of first administration of the investigational product; use of medication that would affect drug metabolism within 28 days of the first administration of the investigational product, or use of any medication that could affect the study results within 10 days of first administration of the investigational product.
Blood sample collection and bioanalysis
Blood samples for pharmacokinetic (PK) analysis were collected into heparinized vacutainer tube (atorvastatin: 7 mL, metformin: 4 mL). In Study 1, Sampling points for the atorvastatin and 2-OH atorvastatin were: pre-dose (0 hour, just before administration), 0.33, 0.66, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 36 hours post-dose. Sampling points for the metformin were: pre-dose (0 hour), 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 24 hours post-dose. Based on results from Study 1, sampling points for the atorvastatin and 2-OH atorvastatin were adjusted in Study 2: pre-dose (0 hour, just before administration), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 14, 24, and 32 hours post-dose. Each blood sample was then centrifuged for 10 min at 2,000 g. The plasma part was transferred into micro-centrifuge vials and stored frozen at −70°C or less until the PK analysis was made.

Sample preparation was performed by liquid phase extraction for atorvastatin and 2-OH-atorvastatin, and by protein precipitation for metformin. Analyte was detected by validated LC-MS/MS methods.[14, 15] The lower limit of quantification (LLQ) for the plasma assay was 0.2 ng/mL for atorvastatin and 2-OH atorvastatin and 10 ng/mL for metformin. The linear calibration range was 0.2-100 ng/mL for atorvastatin and 2-OH atorvastatin and 10-3,000 ng/mL for metformin. The overall precision for atorvastatin and 2-OH atorvastatin ranged from 1.4% to 4.7% and 1.4% to 4.0%, respectively. The overall accuracy for atorvastatin and 2-OH atorvastatin ranged from 91.5% to 109.5% and 92.3% to 105.2%, respectively. The overall precision and accuracy for metformin ranged from 0.6% to 2.3% and 96.6% to 105.1%, respectively.

Pharmacokinetic assessment
The PK variables for atorvastatin, 2-OH atorvastatin and metformin were assessed by non-compartmental analysis using WinNonlin® (ver 6.1; Pharsight Corp., Cary, NC, USA). The AUC0–t and Cmax were determined using the linear trapezoidal method up to the last point on the plasma concentration-time curve where the measured plasma concentration was greater than the LLQ. The Cmax and the time to reach Cmax (Tmax) were determined from the plasma concentration–time curve. AUC0–t was estimated as the sum of AUC to the last measurable concentration and the extrapolated area given by the quotient of the last measurable concentration and terminal elimination rate constant. Below the LLQ values occurring before Tmax were treated as zero, and those occurring after Tmax were excluded from PK analysis.

Safety assessment
A safety assessment was carried out on all subjects who treated. Any adverse event (AE) was reviewed by integrating data from vital signs, clinical laboratory tests, physical examinations, and patient interviews.

Statistical analysis
PK variables were summarized using descriptive statistics. Log-transformed AUC0–t and Cmax were analyzed using analysis of variance (ANOVA) with period, sequence and treatment as fixed effect and subject within sequence as a random effect. The point estimate and the 90% IC for the FDC-to-IC GMR were estimated for each PK variable after gaining mean squared error from ANOVA. Bioequivalence was established if the 90% CIs for AUC0–t and Cmax for both atorvastatin and metformin were within the interval of 0.8 to 1.25. McNemar test was conducted to compare the percentage of AE between treatment groups. Statistical analysis was performed using SAS® software (ver 9.4; SAS Institute Inc., Cary, NC).

Results
Subjects characteristics
A total of 42 subjects were randomized in Study 1; 36 subjects completed the study, and six subjects were discontinued. Reasons for discontinuation were protocol violation (n=2), withdrawal of consent (n=3), and investigator discretion (n=1; not treated). Forty-two subjects were randomized in Study 2; 39 subjects completed the study, and 3 subjects were dropped out due to investigator’s discretion (n=1) and withdrawal of consent (n=2). Mean (SD) age was 24.1 (3.19) and 24.0 (2.10) years, and mean (SD) body mass index was 22.2 (2.08) and 22.4 (1.88) kg/m2 in Study 1 and Study 2, respectively. In each study, demographics and baseline characteristics were similar across both sequence groups.

Pharmacokinetics
The plasma concentration versus time profiles obtained for atorvastatin, 2-OH atorvastatin and metformin after administration of the FDC and IC to healthy subjects in two different periods were very similar (Fig. 1 and 2). The PK variables for atorvastatin and metformin are summarized in table 1.

The Cmax and AUC0–t for atorvastatin and metformin were used to assess bioequivalence between the FDC tablet and IC tablets. In study 1, for atorvastatin the GMRs (90% CIs) for Cmax and AUC0–t were 0.906 (0.818-1.005) and 0.911 (0.856-0.969), respectively. The GMRs (90% CIs) obtained for metformin for Cmax and AUC0–t were 1.130 (1.076-1.187) and 1.010 (0.962-1.060), respectively. In study 2, the GMRs (90% CIs) for atorvastatin for Cmax and AUC0–t were 0.974 (0.873-1.086) and 0.972 (0.915-1.032), respectively. The GMRs (90% CIs) obtained for metformin for Cmax and AUC0–t were 1.068 (1.019-1.119) and 0.955 (0.927-0.983), respectively. The results of the statistical analysis showed that the 90% CIs for atorvastatin and metformin were within the bioequivalence acceptance criteria of 0.8-1.25 (Fig. 3).

Safety and Tolerability
AE profiles were similar for the FDC and the IC treatment, irrespective of dosage strength (McNemar test; p=0.4795 in study 1 and 1 in study 2). No serious AEs and subject withdrawn due
**Table 1.** Summary statistics of atorvastatin, 2-OH atorvastatin and metformin pharmacokinetic variables following a single oral administration

|                  | Study 1 (n=36) |                  | Study 2 (n=39) |
|------------------|----------------|-----------------|----------------|
|                  | CJ-30056 20/500 mg | Lipitor® 20 mg + Glucophage® XR 500 mg | CJ-30056 20/750 mg | Lipitor® 20 mg + Glucophage® SR 750 mg |
| **Atorvastatin** |                |                  |                |
| AUC<sub>0-t</sub> (ng·hr/mL) | 51.56 ± 27.489 | 55.12 ± 26.416 | 40.52 ± 20.272 | 41.71 ± 21.964 |
| AUC<sub>0-∞</sub> (ng·hr/mL) | 56.13 ± 28.582 | 59.49 ± 27.590 | 44.54 ± 20.551 | 45.63 ± 22.642 |
| C<sub>max</sub> (ng/mL) | 6.95 ± 4.222 | 7.36 ± 3.577 | 5.83 ± 3.085 | 6.03 ± 2.883 |
| T<sub>max</sub> (hr)<sup>§</sup> | 5.00 (1.00-6.00) | 4.00 (1.50-6.00) | 3.00 (1.00-5.00) | 4.00 (0.50-8.00) |
| t1/2 β (hr) | 8.73 ± 2.742 | 8.44 ± 2.308 | 9.45 ± 2.551 | 9.11 ± 2.661 |
| **2-OH atorvastatin** |                |                  |                |
| AUC<sub>0-t</sub> (ng·hr/mL) | 72.91 ± 27.622 | 77.09 ± 28.513 | 52.51 ± 21.728 | 50.74 ± 19.555 |
| AUC<sub>0-∞</sub> (ng·hr/mL) | 79.91 ± 29.417 | 83.17 ± 30.867 | 59.11 ± 23.200 | 57.40 ± 21.620 |
| C<sub>max</sub> (ng/mL) | 6.31 ± 2.614 | 6.55 ± 2.567 | 5.28 ± 2.875 | 4.74 ± 2.134 |
| T<sub>max</sub> (hr)<sup>§</sup> | 5.00 (4.00-8.00) | 5.00 (3.00-12.00) | 5.00 (3.00-6.00) | 5.00 (3.00-10.00) |
| t1/2 β (hr) | 9.38 ± 1.642 | 9.02 ± 1.220 | 10.22 ± 2.710 | 9.89 ± 2.747 |
| **Metformin** |                |                  |                |
| AUC<sub>0-t</sub> (ng·hr/mL) | 5842.94 ± 1167.450 | 5741.64 ± 986.618 | 7346.15 ± 1622.352 | 7641.45 ± 1345.975 |
| AUC<sub>0-∞</sub> (ng·hr/mL) | 5994.09 ± 1181.064 | 5933.68 ± 990.071 | 7560.75 ± 1665.366 | 7880.47 ± 1398.288 |
| C<sub>max</sub> (ng/mL) | 612.47 ± 108.518 | 541.81 ± 94.140 | 820.42 ± 165.231 | 763.40 ± 130.669 |
| T<sub>max</sub> (hr)<sup>§</sup> | 6.00 (4.00-10.00) | 6.00 (5.00-10.00) | 5.00 (4.00-10.00) | 5.00 (4.00-10.00) |
| t1/2 β (hr) | 4.01 ± 0.714 | 4.06 ± 0.662 | 4.52 ± 0.577 | 4.18 ± 0.791 |

AUC<sub>0-t</sub>, area under the plasma concentration-time curve from time 0 to last measurable concentration; AUC<sub>0-∞</sub>, area under the plasma concentration-time curve from time 0 to infinity; C<sub>max</sub>, maximum plasma concentration; T<sub>max</sub>, time to maximum concentration; t1/2 β, terminal elimination half-life; § values expressed as median (range).

Figure. 1 Mean (±SD) plasma atorvastatin (a), 2-OH atorvastatin (b) and metformin (c) concentration-time profiles 3 after a single oral administration of atorvastatin/metformin 20/500 mg given as the fixed-dose combination tablet 4 or individual component tablets under fed conditions in study 1 (n=36). Dashed horizontal lines show the lower 5 limit of quantification (0.2 ng/mL for both atorvastatin and 2-OH atorvastatin; 10 ng/mL for metformin). Values 6 below the limit of quantification were entered as 0 and included as such in the calculation of means.
In study 1, three AEs (headache [1], influenza like illness [1] and oropharyngeal pain [1]) were reported for two (4.9%) subjects after administration of the FDC tablet and none after the IC tablets. All AEs were mild in severity and resolved without sequelae. In study 2, two AEs (creatine phosphokinase increased [2]) were reported for two (4.8%) subjects after administration of the FDC tablet and two AEs (creatine phosphokinase increased [1] and headache [1]) for 2 (4.8%) subjects after the IC tablets. All AEs were moderate to severe in severity and resolved without sequelae.

No other clinically significant hematology or biochemistry laboratory abnormalities, vital signs, or electrocardiogram abnormalities were reported in both of the studies.

Discussion

The present studies demonstrated that two dosages strengths of the newly developed FDC tablets and corresponding IC tablets have comparable PK characteristics in healthy adult male subjects. Both treatments indicated good tolerance in this population, and no serious AEs were observed.

Active tubular secretion in the kidney is the major route of elimination of metformin, while atorvastatin undergoes extensive first-pass metabolism in the liver and small intestine.[16,17] The metabolic pathway of atorvastatin is complex with cytochrome P450 3A4-mediated oxidation, beta-oxidation, lactonization, hydrolysis and intestinal-mediated glucuronidation.[17] About 70% of the circulating lipid lowering activity is attributable to 2-OH atorvastatin, which is equipotent to the parent drug in vitro.[18] If metabolite may be formed as a result of gut wall or pre-systemic metabolism, it is recommended that both the metabolite and the parent drug be measured. PKs of 2-OH atorvastatin were comparable between FDC tablets and IC tablets in these studies, and these findings can be used to provide supportive evidence of comparable therapeutic outcome.

Multiple drug therapy is very common in patients with diabetes. A combined therapy of an anti-hyperglycemic agent and a statin is indicated for the treatment of diabetes patients with a risk of CHD.[4,5] However, polypharmacy and complexity of treatment regimen are known to be two of the determinants of poor medication compliance in chronic disease management.
[19] FDC have been shown to be superior in compliance and the risk of non-compliance to medication regimen is reduced by 24-26% with FDC.[20]

In conclusion, newly developed CJ-30056 20/500 mg and CJ-30056 20/750 mg FDC tablets are expected to be used as alternatives to IC tablets to decrease the pill burden and increase treatment compliance in diabetic patients with a risk of CHD.

Acknowledgements

These studies were sponsored by CJ HealthCare Co., Ltd. The authors wish to thank the clinical site staff and subjects for their contributions to these studies.

Conflicts of interests

- Authors: There is no conflict of interests.
- Reviewers: Nothing to declare
- Editors: Nothing to declare

References

1. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al., Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366:1267-1278.
2. Singh IM, Shishehbor MH, Ansell BJ. High-density lipoprotein as a therapeutic target: a systematic review. JAMA 2007;298:786-798.
3. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Treatment of High Blood Cholesterol in, Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285: 2486-2497.
4. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, et al., Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet 2004; 364:685-696.
5. Collins R, Armitage J, Parish S, Sleigh P, Peto R, Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 2003;361:2005-2016.
6. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulphonylurea mono-therapy in type 2 diabetes. Diabetes Care 2002;25:2244-2248.
7. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-865.
8. Zangiabadi N, Shafiee K, Alavi KH, Assadi AR, Damavandi M. Atorvastatin treatment improves diabetic polyneuropathy electrophysiological changes in non-insulin dependent diabetic patients: a double blind, randomized clinical trial. Minerva Endocrinol 2012;37:195-200.
9. Athyros VG, Karagiannis A, Katsiki N, Mihalidis DP. Relation of improvement in glomerular filtration rate with atorvastatin to reductions in heart failure morbidity. Am J Cardiol 2012;110:763. doi:10.1016/j.amjcard.2012.06.007.
10. Lehman DM, Lorenzo C, Hernandez J, Wang CP. Statin use as a moderator of metformin effect on risk for prostate cancer among type 2 diabetic patients. Diabetes Care 2012;35:1002-1007.
11. Scheen AJ. Drug interactions of clinical importance with antihyperglycaemic agents: an update. Drug Saf 2005;28:601-631.
12. Oh JH, Eun Lee J, Jeong Kim Y, Oh TO, Han S, Jeon EK, et al., Designing of the fixed-dose gastroretentive bilayer tablet for sustained release of metformin and immediate release of atorvastatin. Drug Dev Ind Pharm 2016;42:340-349. doi: 10.3109/03639045.2015.1096279.
13. Liu YM, Pu HH, Liu GY, Jia JY, Weng LP, Xu RJ, et al., Pharmacokinetics and bioequivalence evaluation of two different atorvastatin calcium 10-mg tablets: A single-dose, randomized-sequence, open-label, two-period crossover study in healthy fasted Chinese adult males. Clin Ther 2010;32:1396-1407. doi: 10.1016/j.clinthera.2010.07.004.
14. Wang Y, Tang Y, Gu J, Fawcett JP, Bai X. Rapid and sensitive liquid chromatography-tandem mass spectrometric method for the quantitation of metformin in human plasma. J Chromatogr B Analyst Biomed Life Sci 2004;808:215-219.
15. He BX, Shi L, Qiu J, Zeng XH, Tao L, Li R, et al. Quantitative determination of atorvastatin and ortho-hydroxy atorvastatin in human plasma by liquid chromatography tandem mass spectrometry and pharmacokinetic evaluation. Methods Find Exp Clin Pharmacol 2013;32: 481-487.
16. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012;22:820-827. doi: 10.1097/FPC.0b013e32835 59b22.
17. Lennermas H. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet 2003;42:1141-1160.
18. Lea AF, McTavish D. Atorvastatin. A review of its pharmacology and therapeutic potential in the management of hyperlipidaemias. Drugs 1997;53: 828-847.
19. García-Pérez LE, Alvarez M, Dilla T, Gil-Guilén V, Orozco-Beltrán D. Adherence to therapies in patients with type 2 diabetes. Diabetes Ther 2013;4:175-194. doi: 10.1007/s13300-013-0034-y.
20. Bangalore S, Kalalakkannan G, Parkar S, Messerli FH. Fixed-dose combinations improve medication compliance: a meta-analysis. Am J Med 2007;120:713-719.