Bioequivalence of levamlodipine besylate tablets in healthy Chinese subjects: A single-dose and two-period crossover randomized study

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Abstract

Background: Levamlodipine, a calcium channel blocker, is used in treatment of hypertension. To compare the pharmacokinetic parameters between levamlodipine test formulation at a single dose of 5 mg and amlodipine reference formulation at a single dose of 10 mg, the bioequivalence study was carried out.

Methods: A single-dose randomized, open-label, two-period crossover study was designed in healthy Chinese subjects. 48 subjects were divided into fasting and high-fat meal group equally. The subjects randomly received the test or reference formulations at the rate of 1:1. Following a 21-day washout period, the alternative formulations were received. The blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 24, 36, 48, 72, 96, 120, 144, 168 hours later. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was applied to determine levamlodipine in the plasma samples.

Results: Within equivalence limits between 80 ~ 125%, the test formulation and the reference formulation were bioequivalent, with the 90% confidence intervals (CIs) for the ratio of geometric means of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$. The data were shown as C_{max} (89.59% ~ 101.61%), $AUC_{0\text{-}t}$ (87.83% ~ 94.87%) and $AUC_{0\text{-}\infty}$ (86.28% ~ 93.49%) under fasting condition, C_{max} (90.93% ~ 102.37%), AUC_{0-t} (95.75% ~ 104.93%) and $AUC_{0-\infty}$ (95.36% ~ 105.33%) under high-fat meal condition. Serious adverse event was not observed.

Conclusions: The trial confirmed that levamlodipine at a single dose of 5 mg and amlodipine at a single dose of 10 mg were bioequivalent under both fasting condition and high-fat meal condition.

Trial registration: Cinicaltrials, NCT04411875. Registered 3 June 2020 - Retrospectively registered, https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S0009W1Q&selectaction=Edit&uid=U00050YQ&ts=3&cx=-6iqkm8

Background

Hypertension is one of the most common risk factors associated with cardiovascular disease, stroke, and can lead to severe complications [1]. Amlodipine, a dihydropyridine calcium antagonist, has therapeutic effect on hypertension and angina pectoris [2]. Amlodipine is an racemic mixture, including (R) and (S)-amlodipine, but only the latter has therapeutic activity [3]. By blocking the L-type calcium channel, (S)-amlodipine, known as levamlodipine, can reduce the influx of calcium into vascular and cardiac smooth muscle cells, resulting in vasodilation and decreased blood pressure [4, 5].

Pharmacokinetics of levamlodipine besylate 2.5-mg tablet in healthy male subjects was studied previously [6]. However, pharmacokinetic studies of other doses of levamlodipine have not been fully carried out yet. The bioequivalence study was designed to compare the pharmacokinetic parameters between levamlodipine test formulation at a single dose of 5 mg and amlodipine reference formulation at a single dose of 10 mg.

Methods

Ethics

The trial was performed abiding by the Declaration of Helsinki [7], Good clinical practice (GCP) [8] and the guidelines of China National Medical Products Administration (NMPA). Relevant documents, including protocol, informed consent and drug inspection report were all approved independently by the Medical Ethics Committee of the Affiliated Hospital of Qingdao University. All protocol violations occurred have been reported to the Medical Ethics Committee.

Subjects

The inclusion criteria for the volunteers included as follows: 1) Healthy male or female aged 18 and above. 2) The body mass index is in the range of 18.6-28.5 kg/m^2 (including the critical value). The weight of male is not less than 50.0 kg, and that of female is not less than 45.0 kg. 3) The following examination show that the indicators are normal or abnormal without clinical significance. The examination including: Vital signs, physical examination, blood routine, blood biochemistry, urinalysis, pregnancy test for female, serological tests for hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV), and syphilis virus, 12 lead ECG, breath test for alcohol, drug abuse test. 4) The subjects have no family planning within 3 months and could select contraceptive method. 5) Before the study, all subjects have been informed of the study's purpose, protocal, benefits, and risks, and signed the informed consent voluntarily.

The exclusion criteria for the volunteers included as follows: Being allergy to the study medications, smoking, alcohol abuse; and participation in another clinical trial within 3 months.

Study design

The single-dose randomized, open-label, two-period crossover study was executed in the Phase I Clinical Research Center of the Affiliated Hospital of Qingdao University. According to the random table generate by SAS 9.4, the subjects were divided into two groups at the ratio of 1:1 (Table 1). The select qualified volunteers were hospitalized in the Phase I Clinical Research Center, and fasted for 10 hours overnight until administration. The medicine was swallowed with 240 ml water at room temperature. Blood samples were taken before administration and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 24, 36, 48, 72, 96, 120, 144, 168 hours after administration. The samples were centrifuged at 1,800 g for 10 min at 4 °C to separate the plasma. The plasma samples were divided into two aliquots and stored at -80 °C until bioanalysis. The half-life of levamlodipine is 30 ~ 50 hours. Washout period, the interval between two administration, is 21 days. In the two periods, the operation was kept the same.

Moreover, in high fat meal group, the high-fat breakfast was arranged within half an hour before taking the medicine. Other procedures were the same as those in the fasting group.

Safety assessment

The safety of levamlodipine besylate tablets was assessed by monitoring vital signs and laboratory tests. Vital signs, such as body temperature, blood pressure, and heart rate, were measured before administration and at 3, 8, 24, 36, 48, 72, 96, 120, 144, 168 hours after that. Before discharge, the subjects were evaluated with blood routine, blood biochemistry, urinalysis, pregnancy test for female, and 12 lead ECG. For adverse events (AEs), clinical symptoms, severity, occurrence and ending time, duration, treatment measures and the correlation with the drugs were recorded. All of the AEs that occur within the 7 half lives of the drug (a half-life 30-50 hours of levamlodipine) were recorded and followed up, unless the subjects returned to normal or stable, or failed to visit.

Bioanalysis

The analysts were blinded to the randomization. Plasma samples were determined by the liquid chromatography-tandem mass spectrometry (LC-MS/MS), which was tested by Suzhou Shenglin Pharmaceutical Technology Co., Ltd. An ACQUITY ultra-high-performance liquid chromatography unit (SHIMADZU, Nexera UHPLC LC-30A, Japan) and a mass spectrometer (Applied Biosystems, MDS Sciex, Triple Quad 6500 plus, Concord, Canada) was used in the study. Under multiple reaction monitoring, LC-MS/MS system adopts positive ionization mode. Data collection and handling was employed with Analyst 1.6.3 software (Applied Biosystems, Foster City, CA, U.S.A.). The solid-phase extraction (SPE) experiments [9] were performed by HLB 96-well Plate (Waters Oasis, WAT058951). The plate contains a reversed phase functionalized polymeric sorbent (30 mg/well), in which particle size is 30 μm. Scheme 1 summarized several steps about the cleaning and extraction process. (a) The cartridges were activated by 800 μL of methanol and 800 μL of water followed. (b) The column was loaded with 150 μL of plasma sample or calibrator, 50 μL of internal standard working fluid (5 ng/mL) and 100 μL of deionized water. (c) 500 μL of deionized water twice and methanol (80%) followed were forced to pass through the column to to achieve depolarization. (d) Levamlodipine was eluted from the column by 500 μL of pure acetonitrile twice. Then the solution was blown dry by a pure N2 stream. 150 μL of pure acetonitrile was add into each sample, and mixed at room temperature for 10 minutes. Ultimately, 20 μL of the extracted sample was injected into the LC MS/MS system.

The method is verified fully by selectivity, accuracy, precision, calibration curve and stability. The drug concentration was linear in the range of 0.0500 ~ 10.0 μg × L^{-1}. The lower limit of quantification was 0.05 μg × L^{-1}, and the standard curve was Y = 0.55667X - 0.0030182 (r^2 = 0.9949). The intra- and interday maximum precision was 5.4% and 4.8%, respectively. The intra- and interday accuracy was -6.7 ~ 3.9% and -3.3 ~ 3.3%, respectively. The extraction recovery of levamlodipine was 94.7 ± 3.9%. There was no significant interference in selectivity or stability.

Pharmacokinetic analysis

All subjects completed the study and were included in the pharmacokinetic analysis. The pharmacokinetic parameters were calculated according to non-compartment model using Phoenix™ WinNonlin® 8.0 software (Pharsight, St. Louis, MO, USA). The value below the lower limit of quantification

that occur before the first measurable concentration are set as zero. Subsequent values below the lower limit of quantification were excluded. Linear trapezoidal rule is applied in AUC_{0-t} calculation. $AUC_{0-\infty}$ is the sum of AUC_{0-t} and the ratio of the last measurable concentration (C_t) to the elimination rate constant (Ke). The maximum plasma concentration (C_{max}) and the time to C_{max} (T_{max}) can be obtained from the concentration-time curve. Elimination half-life, $T_{1/2} = 0.693/Ke$.

Statistical analysis

Analysis of variance (ANOVA) was performed on the ln-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ to assess the effects from subject, treatment, period, and preparation. Statistical data were presented as mean ± standard deviation (SD). The probability value less than 0.05 is considered statistically significant. Calculate the 90% confidence intervals (CIs) of the geometric mean ratio of the main indicators. If it is within the equivalent range (80 ~ 125%), it is judged as bioequivalence, and the results of double unilateral t-test are listed. T_{max} was analyzed by non-parametric statistical test. Statistical analyses were performed by SAS 9.4 (SAS Institute Inc. Cary, NC, USA).

Results

Characteristics of the subjects

All of subjects completed the study. A total of 24 subjects including 6 women and 18 men enrolled in fasting group. Among them, 23 were Han and 1 was Manchu nationality. Parameter, mean ± SD (range): age, 31.04 ± 8.04 years (19.00 ~ 48.00 years); weight, 62.06 ± 8.59 kg (48.00 ~ 83.00 kg); height, 168.27 ± 7.97 cm (152.00 ~ 185.00 cm); BMI, 21.90 ± 2.57 kg × m^{-2} (19.00 ~ 27.90 kg × m^{-2}). A total of 24 subjects including 6 women and 18 men enrolled in high fat meal group. All of them were Han nationality. Parameter, mean ± SD (range): age, 32.46 ± 10.45 years (18.00 ~ 51.00 years); weight, 66.88 ± 8.8 kg (50.00 ~ 81.00 kg); height, 167.77 ± 6.63 cm (154.00 ~ 178.00 cm); body mass index (BMI), 23.76 ± 2.86 kg × m^{-2} (18.90 ~ 28.30 kg × m^{-2}).

Pharmacokinetics

Following a single dose of test formulations or reference formulations, individual plasma concentration-time curves were presented in Figure 1 (fasting group) and Figure 2 (high fat meal group). The mean plasma concentration-time curves of the two levamlodipine formulations were shown in Figure 3 (fasting group) and Figure 4 (high fat meal group).

Mean pharmacokinetic parameters from the fasting group (Table 2) and the high fat meal group (Table 3) are summarized. In the fasting group (Table 4), ANOVA indicated a lack of effects from treatment for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. There was a significant difference from subjects for C_{max} ($p \leq 0.05$). There was a significant difference from formulations and preparations for AUC_{0-t} ($p \leq 0.05$) and $AUC_{0-\infty}$ ($p \leq 0.05$). In the high fat meal group (Table 5), ANOVA indicated a lack of effects from treatment and preparation

for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. There was a significant difference from subjects for C_{max} ($p \leq 0.05$), AUC_{0-t} ($p \leq 0.05$), and $AUC_{0-\infty}$ ($p \leq 0.05$). There was a significant difference from periods for AUC_{0-t} ($p \leq 0.05$).

The 90% CIs for the geometric mean ratios of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and the power are presented in Table 6 (fasting group) and Table 7 (high fat meal group). These ratios were within the predefined equivalence limit of 0.80 ~ 1.25.

Safety

During the whole study period, both test preparation and reference preparation showed good tolerance. The AEs found in physical examination, electrocardiogram (ECG) and laboratory examination were listed in Table 8 (fasting group) and Table 9 (high-fat diet group). None of them were judged as serious adverse events (SAEs).

Discussion

The present study was performed to compare the pharmacokinetics of a newly-developed levamlodipine besylate tablet at a single dose of 5 mg (test formulation, anglikang Pharmaceutical Company Co., Ltd., Zhejiang, China) with that of a marketed amlodipine besylate tablet at a single dose of 10 mg (reference formulation, Pfizer Pharmaceuticals Limited, USA) for assessment of bioequivalence in healthy Chinese volunteers under fasting and high fat meal conditions.

Amlodipine besylate tablets were developed by Pfizer Pharmaceutical Co., Ltd. The active component of amlodipine is racemate, in which the ratio of (R)-amlodipine to (S)-amlodipine is 1:1. The optimal isomer is (S)-amlodipine, also known as levamlodipine. In vivo, there is no mutual transformation between (R)-amlodipine and (S)-amlodipine. Before the start of this study (November 13, 2019),there is no approved levamlodipine besylate tablet on the market in the European Union, the United States, Japan, and China. Moreover, the pharmaceutical research of the the new developed test formulation is carried out according to the amlodipine besylate tablets developed by Pfizer. Combined with Chinese drug consistency evaluation catalogue, amlodipine besylate tablets (Norvasc®) produced by Pfizer was selected as the reference formulation in this study. On December19, 2019, levamlodipine maleate tablets (Conjupri®) produced by CSPC Ouyi pharmaceutical Co., Ltd. was granted FDA approval. However, the acid radical of this product is different from the test formulation in this study.

In this study, the parameters such as recoveries, matrix effects, linear range, lower limit of quantification, stability by specificity, precision, and accuracy specifications, were investigated to confirm the method of LC-MS/MS. Concentration of levamlodipine in human plasma showed good linear relationship within 0.05 ~ 10 $\mu g \times L^{-1}$. The relative standard deviation (RSD) values of intra- and interday precision were both less than 10%; and endogenous substances do not interfere with the determination of levamlodipine [9]. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were transformed by logarithm and analyzed by variance. The 90% CIs for the geometric mean ratios of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and the power is in the equivalent interval of 0.80 ~ 1.25.

According to FDA bioequivalence guidance, the final elimination half-life of amlodipine is about 30 ~ 50 hours. Thus, washout period is set to 21 days, which is a length of time greater than seven half lives. And the blood samples taken for 168 hours was enough.

In view of the inhibitory effect of amlodipine on CYP3A4 [10], subjects with behaviors of drinking, smoking and drug intake through CYP3A4 metabolism were excluded. During the trial, the use of drugs metabolized through CYP3A4 did not happen. In this open-label study, AEs assessment may not be objective enough. After the test formulation is approved for marketing, the efficacy and side effects need to be further explored.

Conclusions

The trial confirmed that levamlodipine at a single dose of 5 mg and amlodipine at a single dose of 10 mg were bioequivalent under both fasting condition and high-fat meal condition. If the test drug levamlodipine can be approved by NMPA, it can be used in the treatment of hypertension in clinic.

Abbreviations

Liquid chromatography-tandem mass spectrometry (LC-MS/MS); confidence intervals (CIs); Good clinical practice (GCP); China National Medical Products Administration (NMPA); human immunodeficiency virus (HIV); adverse events (AEs); solid-phase extraction (SPE); area under curve (AUC); maximum plasma concentration (C_{max}); time to C_{max} (T_{max}); elimination half-life ($T_{1/2}$) standard deviation (SD); body mass index (BMI); electrocardiogram (ECG); serious adverse events (SAEs); relative standard deviation (RSD).

Declarations

Ethics approval

The study passed the review of Medical Ethics Committee of the Affiliated Hospital of Qingdao University on August 30, 2018, and obtained the approval (No.: QYFYEC 2018-065-01).

Consent for publication

All presentations of reports have been consent for publication.

Availability of data and materials

We have shared the raw data by providing it in a supplementary file.

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Authors' contributions

The study was designed by Yu Cao. Xin Li wrote the paper, participated in data statistics and performance. Chenjing Wang, Ting Li, Yan-ping Liu, Shu-qin Liu, Ye Tao, Ya-ping Ma and Xiao-meng Gao performed research.

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Figures

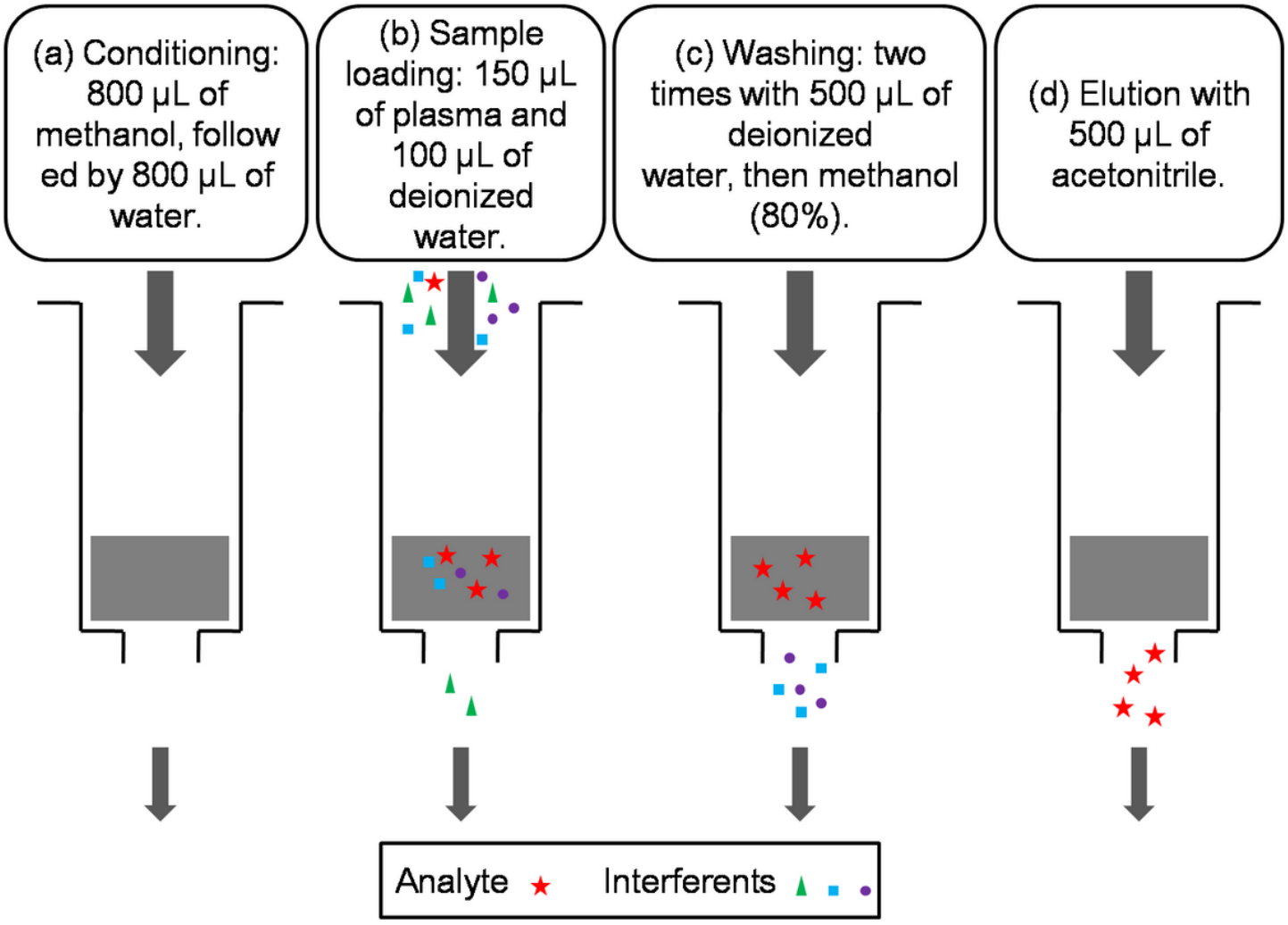

Figure 1

Scheme 1

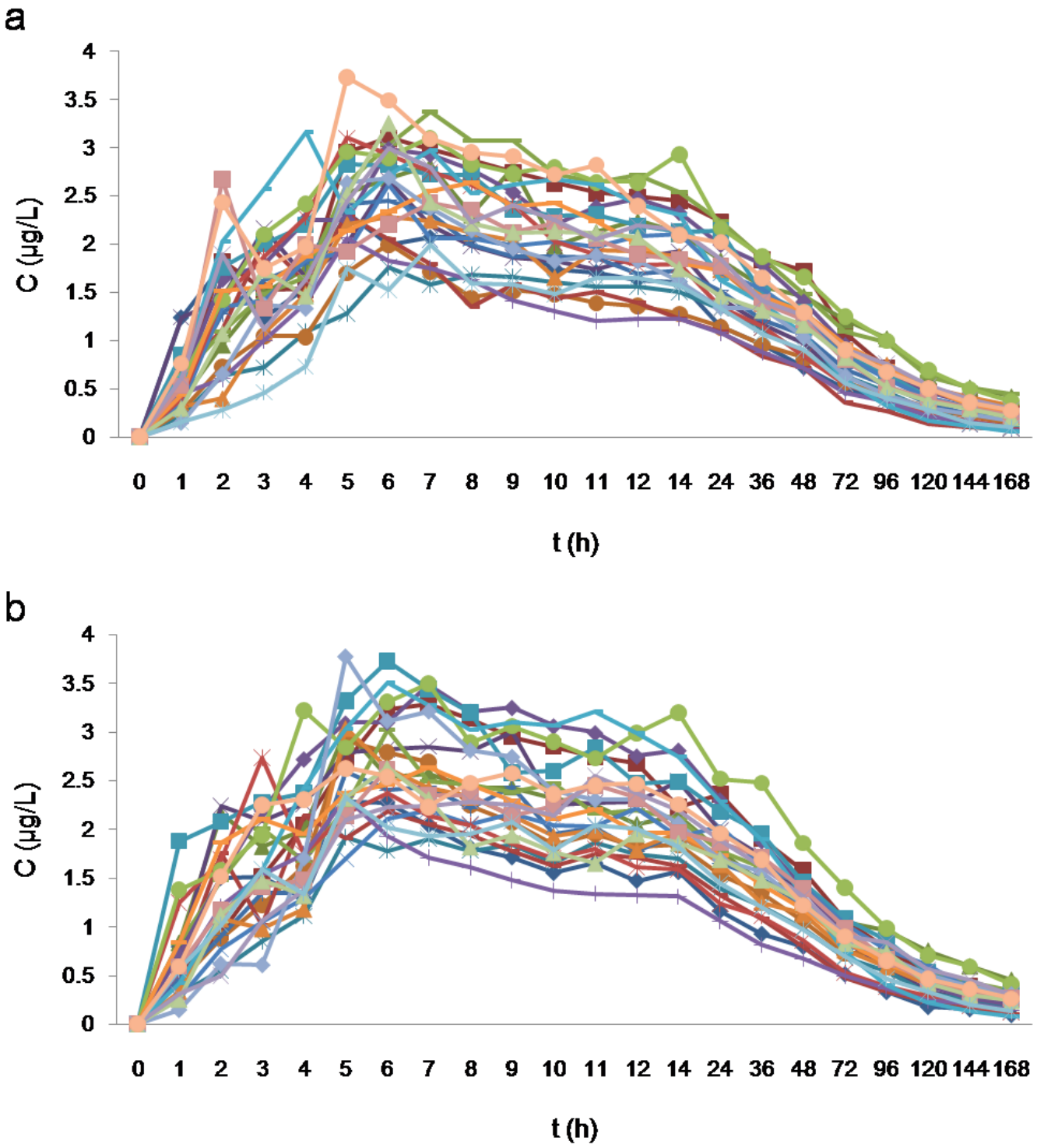

Figure 2

Figure 1

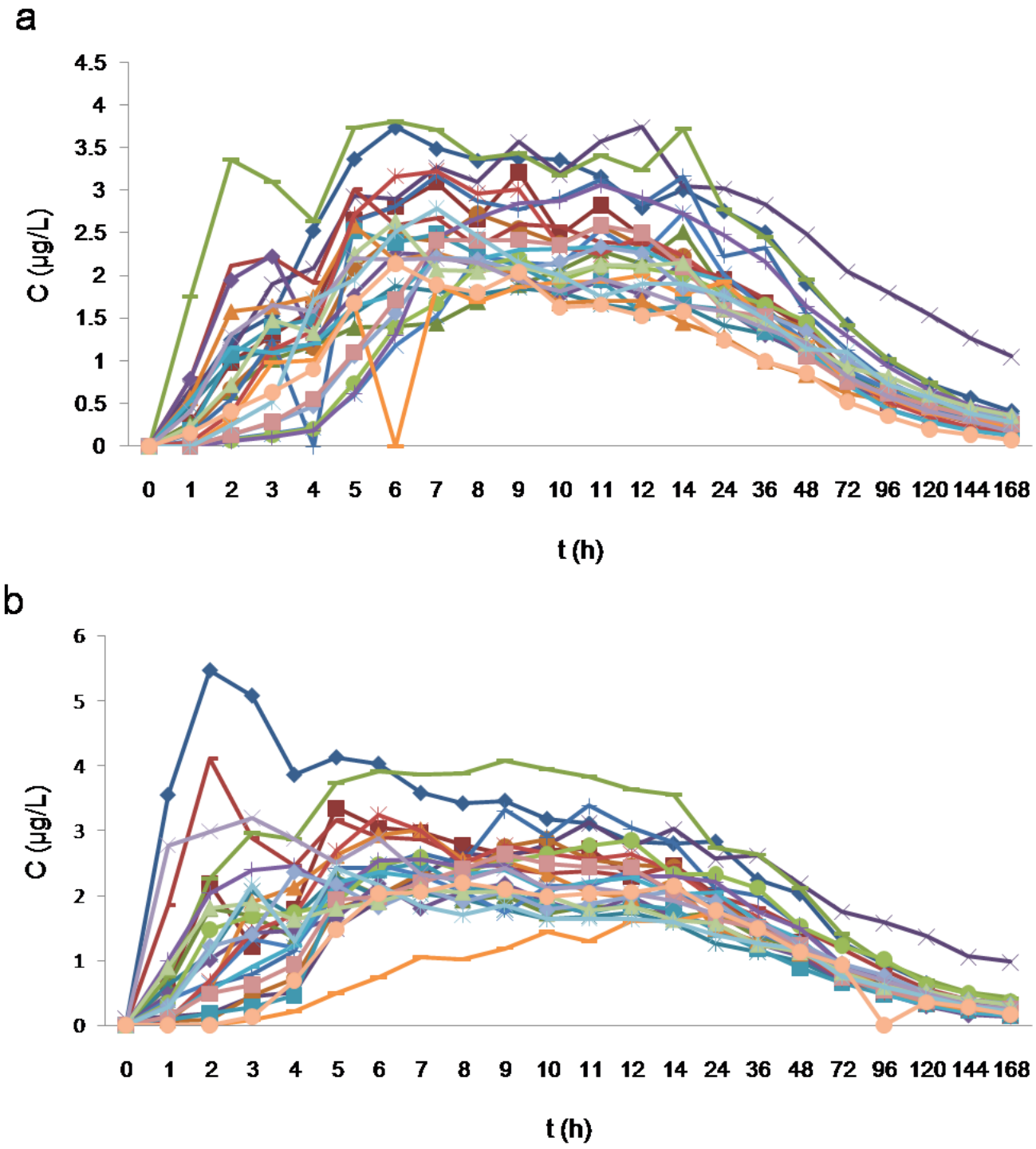

Figure 3

Figure 2

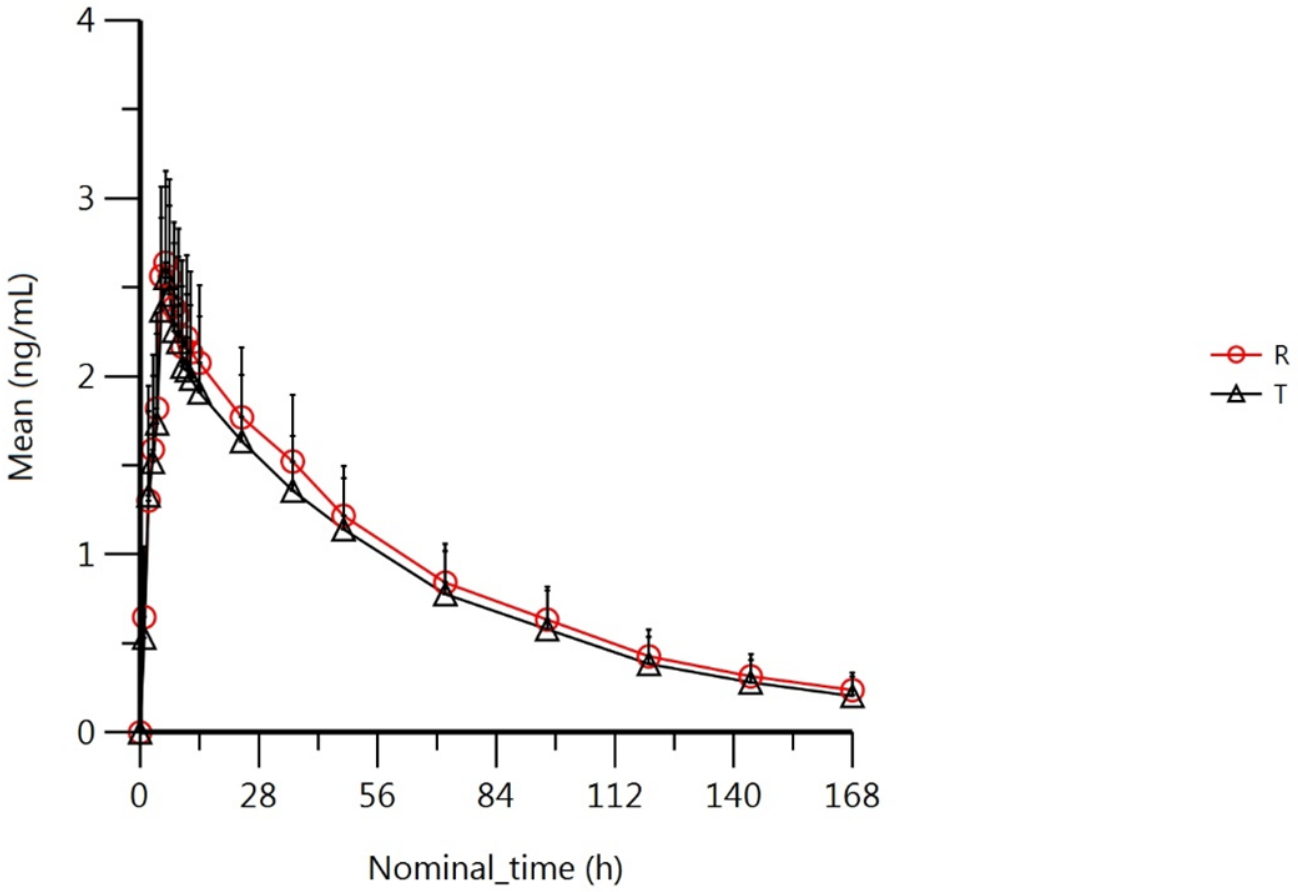

Figure 4

Figure 3

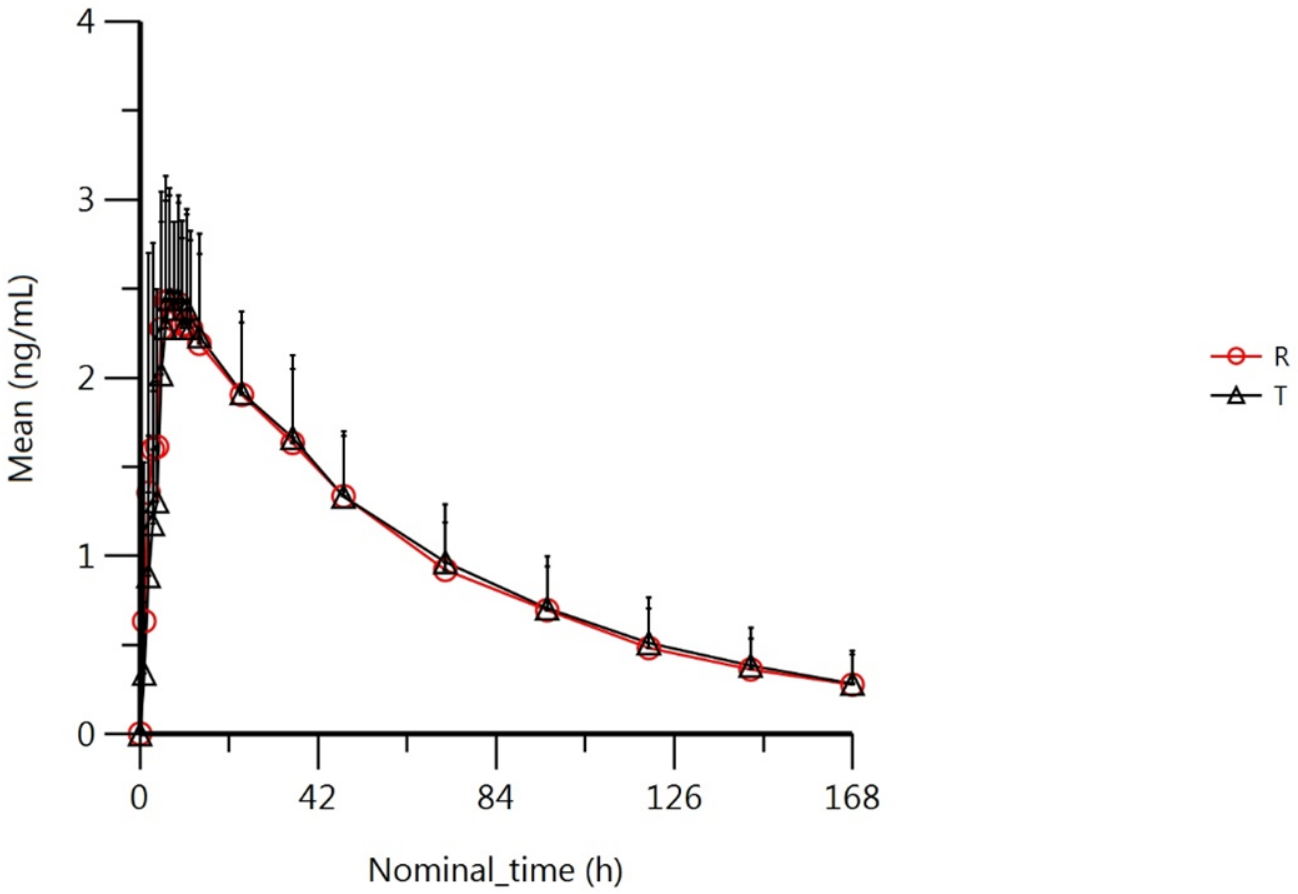

Figure 5

Figure 4

Supplementary Files

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