Pharmacokinetics and Bioequivalence Study of Two Indapamide Formulations after Single-dose Administration in Healthy Chinese Male Volunteers

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Key words
- indapamide
- pharmacokinetics
- bioequivalence
- healthy volunteers
- HPLC-MS
- human plasma

Abstract

The pharmacokinetics and relative bioavailability/bioequivalence of 2 formulations of indapamide were assessed in this paper. The study was conducted in 20 healthy Chinese male volunteers according to an open, randomized, single-blind, 2-way crossover study design with a wash-out phase of 7 days. Blood samples for pharmacokinetic profiling were taken up to 84 h post-dose, and indapamide concentrations in plasma were determined by a validated liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) method. Based on the plasma concentration-time data of each individual in each period, pharmacokinetic parameters, $C_{\text{max}}$, $T_{\text{max}}$, $AUC_0-\infty$, $AUC_{0-\infty}$ and $t_{1/2}$, were calculated by non-compartmental analysis. Pharmacokinetic parameters for test and reference formulations were analyzed statistically to assess bioequivalence of the 2 formulations. The values of $C_{\text{max}}$, $T_{\text{max}}$, $AUC_0-\infty$, $AUC_{0-\infty}$ for test and reference formulations were 49.53 ± 5.53 and 47.79 ± 4.68 ng/mL, 1.9 ± 0.6 and 2.0 ± 0.5 h, 22.49 ± 5.93 and 23.23 ± 4.48 h, 859.51 ± 160.92 and 840.90 ± 170.62 ng·h/mL, 934.35 ± 190.60 and 919.52 ± 179.74 ng·h/mL, respectively. The 90% confidence intervals of the T/R-ratios of logarithmically transformed data were within the accepted range of 80–125%. It showed that the 2 formulations of indapamide were bioequivalent. Both preparations were well tolerated and no adverse reactions were found throughout the study.

Introduction

Indapamide, 4-chloro-N-(2-methyl-1-indolinyl)-3-sulphamoyl benzamide, is a non-thiazide indole derivative of O-chloro benzene sulfonamide, which has diuretic and natriuretic effects and thus exhibits an anti-hypertensive action causing a decrease in systolic, diastolic and mean blood pressure [1, 2]. However, specific side chains endows the drug some characteristic properties. Indapamide presents an indolinyl ring and uniquely possesses free-radical scavenging activity as well as a direct vasodilator action [2–4], besides it has no adverse lipid effects in contrast to thiazide diuretics [5–7]. Accordingly, indapamide was defined by the 1999 WHO/ISH Hypertension Guidelines and JNC VII as a first-line drug for treating mild-to-moderate hypertension [8]. Indapamide was first developed as 2.5 mg immediate release tablets and subsequently 1.5 mg sustained release tablets. Indapamide is quickly absorbed from the gastrointestinal tract and peak plasma concentrations are seen 1–2 h after dosing immediate release tablets. Indapamide is widely distributed throughout the body, with extensive binding to some specific sites, which leads its relatively highly combination to plasma proteins (79%) in plasma [9]. It has a long half-life (about 20 h) so it is effective in once daily dosing [10]. Indapamide is extensively metabolised, with only about 7% of the total dose administered recovered in the urine as unchanged drug during the first 48 h after administration [10, 11]. Although Li Ding et al. [12], Wei-Dong Chen et al. [13] and Tai-Jun Hang et al. [9] have researched the pharmacokinetics and bioavailability of indapamid in the Chinese population, they focus on the method for the quantitation of indapamide in vivo, which is simple, sensitive and rapid. The information concerning the bioavailability and pharmacokinetics of indapamide in the Chinese population has not been reported in detail up to now. In this article, pharmacokinetic parameters of indapamide in Chinese after dosing 5 mg indapamide are presented. Meanwhile, this study also focused on a comparison of the bioavailability of generic drug (test) and original drug (reference) formulations of indapamide of the same
strength (5 mg) produced by 2 different manufacturers. The generic drug product is therapeutically equivalent to the original product and can be used alternatively. Besides, as a way of curbing healthcare expenditure, many countries including China have take measures to boost their generics markets. Therefore, it is of great significance to evaluate bioequivalence of the generic drug reasonably.

Materials and Methods

Materials

Indapamide test tablets (2.5 mg/tablet; Batch No. 20110302) were supplied by Jiangsu Fubang Pharmaceutical Co. Ltd., (Jiangsu, P. R. China); Indapamide reference tablets (2.5 mg/tablet; Batch No. 869000) were obtained from Servier Pharmaceutical Co. Ltd., France. Indapamide standard and prednisone standard (IS) were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P. R. China).

Method validation

The method was validated in our analytical laboratory according to the United States Food and Drug Administration (FDA) bioanalytical method validation guidance [14] on specificity, matrix effect, linearity, accuracy, precision, extraction recovery and stability.

Specificity was assessed by testing 6 different drug-free plasma batches. The matrix effect caused by ionization competition between analytes (indapamide and IS) and co-eluted compounds was evaluated at 3 QC concentrations. 5 calibration curves (1, 2, 5, 10, 20, 50, 100 ng/mL) were generated in 5 consecutive days to test the linearity. The limit of detection (LOD) and the lower limit of quantification (LLOQ) were determined as the concentrations with a signal-to-noise ratio (S/N) no less than 3 and 10, respectively. The accuracy and precision were evaluated by analyzing QC standards 5 times daily and over 3 consecutive days. Extraction recovery was evaluated by comparing the indapamide-IS peak area ratios obtained from extracted samples to those from the samples containing the same amount of indapamide which was added after the extraction step (IS was added after the extraction step in both batches). Stability was evaluated at each QC concentration under different conditions that occurred during sample analysis. The short-term stability and long-term stability was evaluated by keeping QC samples at room temperature for 8 h and low temperature (−20 °C) for 15 days respectively. The post-preparative stability was measured by placing prepared QC samples under the autosampler conditions (15 °C) for 48 h. The freeze and thaw stability was tested by analyzing QC samples undergoing 3 freeze (−20 °C)-thaw (room temperature) cycles on consecutive days. The working solutions and stock solutions of indapamide and the IS were also evaluated at room temperature for 24 h and at 4 °C for 15 days, respectively.

Experimental design

This open, randomized, single-dose, 2-sequence, 2-period crossover study was performed in strict accordance with the Declaration of Helsinki, Good Clinical Practice (GCP) Guidelines and Good Laboratory Practice (GLP) Guidelines. The protocol and the consent form of the study were approved by the Research Ethics Committee of Nanjing Command General Hospital, Nanjing (P. R. China).

Enrolled in this study were 20 healthy, male, adult Chinese volunteers provided written informed consent prior to study participation. All of them completed the study and were evaluable for this pharmacokinetics and bioequivalence study. Their mean age ± SD was 23.60 ± 1.39 years. Their mean body weight was 66.15 ± 7.01 kg and their mean height was 172.85 ± 3.18 cm, giving a mean Body Mass Index (BMI) of 22.11 ± 1.94 kg/m². Screening at study start included medical history, physical examination, vital signs, ECG, laboratory safety tests (routine hematology; glucose, creatinine, sodium, potassium, chloride, CO₂, ALT, ALP, and total bilirubin level; and urinanalysis). Subjects were excluded if they smoked, used alcohol, had a history or evidence of hepatic, renal, gastrointestinal, or hematological deviations, had acute or chronic disease, allergies to any medications, and/or had any clinically significant abnormality. Subjects were instructed to abstain from using any

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medications for at least 2 weeks prior to and during the study; and from smoking and taking alcohol, caffeine, related xanthenes-containing beverages or food for at least 48 h before administering the study drug and throughout the study. This was a single-dose, single-blind, randomized, 2-way crossover trial in which the subjects were randomly assigned to the treatment sequences R/T or T/R. The subjects received a single dose of 5 mg indapamide as 2 tablets of formulation R (indapamide reference tablets) or a single dose of 5 mg indapamide as 2 tablets of formulation T (indapamide test tablets) under supervision by study investigators. The reference formulation and test formulation were administered alternately in the second period. The wash-out period between the 2 dosing periods was 1 week. In each treatment period, volunteers were required to fast for approximately 10 h prior to the dosing. The formulations were administrated with 250 mL water, then water was not allowed until 2 h after administration. Standardized lunch and dinner were served after a 6- and 12-h blood sampling, respectively. For the measurement of pharmacokinetic parameters, venous blood samples were collected by study nurses via an indwelling cannula or direct venipunture into the heparinised tubes from all subjects prior to the dosing (T₀) and then after 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 84 h from administration during each treatment period. Samples were centrifuged at 3000 g for 5 min immediately then the plasma was transferred and stored at −20°C until assay.

Pharmacokinetic and statistical analysis
Pharmacokinetic parameters
Pharmacokinetic assessment of indapamide was estimated from the concentration-time data using non-compartmental methods. Maximum plasma concentrations (C_{max}) and time to reach this value (T_{max}) were considered as the observed highest concentration and time of its occurrence, respectively. The elimination half-life (t_{1/2}) was calculated as t_{1/2} = (ln 2)/Kel, where the apparent elimination rate constant, Kel was calculated as the slope of the linear regression line of natural log-transformed plasma concentration against time. AUC_{0−∞} was calculated according to the following formula: AUC_{0−∞} = τ/Auc_{0−τ} + C_{τ}/Kel. The rate of absorption was evaluated by means of the ratio: C_{max}/AUC_{0−∞}. The ratio AUC_{0−τ}/AUC_{0−∞} was determined as an indicator of the adequacy of the blood sampling period.

Statistical analysis
The pharmacokinetic primary variables for bioequivalence testing in this study were AUC_{0−∞}, AUC_{0−τ}, and C_{max}. An analysis of variance (ANOVA) was performed on these parameters, after natural logarithmic transformation using general linear model procedure for randomized crossover design to evaluate the effects of formulation, period, sequence, and subject (within sequence) at the significance level (α) of 0.05. ANOVA was performed with the statistical software package, SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA), using the General Linear Model (GLM) Procedure. The Wilcoxon signed rank test was used for the non-parametric analysis to determine differences in T_{max}. The sequence effect was tested against the mean squares term of subject (within sequence) at the significance level of 0.05. Effects were considered statistically significant if the probability obtaining the calculated F test was ≤0.05. According to the guidance from the FDA, product bioequivalence was based on the 90% confidence intervals (CIs) for the ratios of least squares means of the ln-transformed values for AUC_{0−∞}, AUC_{0−τ}, and C_{max}. In addition, the bioequivalence between the 2 formulations was assessed by Schurimann’s 2 one-sided t-tests.

Results
Analytical method
The representative chromatograms of indapamide are depicted in ▶Fig. 1a–c. As shown, the retention times of indapamide and IS were 7.8 and 11.4 min, respectively. The total LC-MS analysis time was 13 min per sample. The sensitivity and specificity of the method were proved by testing 6 different plasma batches (▶Fig. 1a). No significant matrix effect for indapamide and the IS was implied in the method. The calibration curve was linear over the concentration range of 1–100 ng/mL, with r^2 > 0.99. LLOQ for indapamide proved to be 1 ng/mL (▶Fig. 1b), and LOD was 0.2 ng/mL. The accuracy and precision at 3 QC levels (2, 20 and 80 ng/mL) are 100.8 ± 10.8%, 102.2 ± 8.2% and 100.9 ± 5.1% for intra-batch, and 100.6 ± 9.1%, 101.4 ± 9.2% and 97.6 ± 6.8% for intra-batch. The accuracy and precision at LLOQ are within (100 ± 20)% and 20%, respectively. The data of extraction efficiency measured for indapamide in human plasma was consistent, precise and reproducible. The results (▶Table 1) showed that both indapamide and the IS were stable under the studied conditions. ▶Table 1 showed the data for intra-batch and inter-batch precision and accuracy of the method, extraction recovery and sample stability test.

Clinical observations
20 subjects completed the study so that 20 cases were available for each treatment. No clinically significant adverse effects were reported in the present study population. All subjects were discharged in good health after repeating physical examination and laboratory analysis after period 2. A clinically relevant difference in both tolerability and safety of 2 treatments was not detected.

Pharmacokinetic and statistical results
The plasma concentration-time profiles of indapamide following single-dose oral administration of the test and reference formulations are presented in ▶Fig. 2. A summary of the pharmacokinetic parameters, AUC_{0−τ}, AUC_{0−∞}, C_{max}, T_{max}, t_{1/2}, MRT, AUC_{0−τ}/AUC_{0−∞}, C_{τ}/Kel and F_{0−∞} derived from the 2 formulations is shown in ▶Table 2. The calculated C_{max} values for the test and reference formulations were 49.53 ± 5.53 ng/mL and 47.79 ± 4.68 ng/mL, AUC_{0−∞} of 934.35 ± 190.60 ng·h/mL (test) and 919.52 ± 179.74 ng·h/mL (reference) were achieved. Elimination half-lives and elimination rate constants were not significantly different between the preparations. In addition, the mean T_{max} values in this study were 1.9 ± 0.6 h for test formulation and 2.0 ± 0.5 h for reference formulation. The results of ANOVA for assessment of treatment, period and subject (within sequence) effects and 90% CIs for the ratio of C_{max}, AUC_{0−τ}, and AUC_{0−∞} values for test and reference formulations, using logarithmic transformed data, are shown in ▶Table 3.
Discussion and Conclusion

The proposed method for determination of indapamide in human plasma was selective, accurate, and precise. And it was proved to be reliable and suitable for the pharmacokinetics and bioequivalence study of indapamide in healthy Chinese male volunteers. In our method, liquid-liquid extraction was necessary and important, because this technique can purify and concentrate...
To exclude the interference of unknown peak to indapamide and specific saturated NaHCO₃ solution were attempted. Finally, 100 μL of the sample. A preliminary evaluation of different extraction solvents such as diethyl ether, ethyl acetate or dichloromethane under alkalizing and neutral conditions showed that extraction with diethyl ether in alkalizing condition exhibited higher extraction efficiency. Then, 1M NaOH, saturated Na₂CO₃ solution and saturated NaHCO₃ solution were attempted. Finally, 100 μL of saturated Na₂CO₃ solution was adopted for sample preparation in terms of precise, consistent and reproducible recoveries and specificity.

To exclude the interference of unknown peak to indapamide peak (showed in Fig. 1c), a mobile phase consisting of 50% methanol and 50% 10 mM ammonium acetate buffer solution (pH=5.0) was adopted, achieved good resolution, symmetric peak shape and suitable retention time without matrix effects. Several articles [15,16] have reported the pharmacokinetics of indapamide in healthy human. Pierre Schiavi [15] researched the pharmacokinetics of sustained and immediate release formulations (1.5 mg and 2.5 mg respectively) of indapamide, indicating much higher values of Cₘₚₓ/dose and AUC₀−∞/dose (39 ± 11 ng/mL and 564 ± 146 ng·h/mL after dosing one 2.5 mg immediate release tablet, determined and calculated by use of concentrations in whole blood) than this study. The main reason should be that indapamide is preferentially and reversibly taken up by the erythrocytes in the blood [17]. Moreover, Dragica Zendelovska [18] developed a new method for determining indapamide in biological fluids to confirm that the Cₘₚₓ in blood was much higher than in serum. Consequently, it should be remarked that blood samples should avoid hemolysis until to obtain the plasma. Moreover, bioequivalence study of indapamide in plasma requires a more sensitive analytical method than in whole blood. LLOQ of 1 ng/mL in this study is enough for plasma concentration determination. In contrast, HPLC-UV methods [9,18], giving a higher LLOQ of 10 ng/mL, are not suitable for pharmacokinetics study of indapamide in plasma. Florin Albu [16] and Deepak S. Jain [19] reported HPLC-MS/MS methods with LLOQ of 1 ng/mL and 0.5 ng/mL respectively, but they adopted a superior instrument (HPLC-MS/MS), which is more expensive than HPLC-MS. Consequently, HPLC-MS is economic and suitable for pharmacokinetics study of indapamide.

The results from the ANOVA found that formulation, period, and sequence had no statistically significant effect on AUC₀−∞, AUC₀−ₜₚ and Cₘₚₓ at the significance level of 0.05. However, as might be expected, the subject variability of those pharmacokinetic parameters was significant (P<0.0001). This was further explored by calculating the inter-subject and intra-subject coefficient of variations. The mean ratio of AUC₀−∞/AUC₀−ₜₚ for test and reference formulations were both above 90%, indicated that the sampling time was adequate. The 90% CIs for AUC₀−∞, AUC₀−ₜₚ and Cₘₚₓ were within the FDA acceptable bioequivalence range of 80–125% and the results of the Schuirmann’s test indicated that the lower and upper limits of the calculated t-test were greater than the critical t-value. Therefore, it can be concluded that the test and reference formulations are bioequivalent according to both of the rate and extent of absorption and consequently can be used interchangeably in

<table>
<thead>
<tr>
<th>Plasma samples</th>
<th>2 ng/mL</th>
<th>20 ng/mL</th>
<th>80 ng/mL</th>
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<tr>
<td></td>
<td>Accuracy (%)</td>
<td>Precision (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>Intra-batch (n=5)</td>
<td>100.8</td>
<td>10.8</td>
<td>102.2</td>
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<tr>
<td>Inter-batch (n=15)</td>
<td>100.6</td>
<td>9.1</td>
<td>101.4</td>
</tr>
<tr>
<td>Extraction recovery</td>
<td>83.2</td>
<td>5.3</td>
<td>82.7</td>
</tr>
<tr>
<td>Long-term stability</td>
<td>97.3</td>
<td>7.3</td>
<td>102.2</td>
</tr>
<tr>
<td>Short-term stability</td>
<td>100.0</td>
<td>3.7</td>
<td>107.4</td>
</tr>
<tr>
<td>Freeze and thaw stability</td>
<td>97.9</td>
<td>6.2</td>
<td>101.3</td>
</tr>
<tr>
<td>Post-preparatory stability</td>
<td>101.0</td>
<td>6.2</td>
<td>104.7</td>
</tr>
</tbody>
</table>

Table 1 Accuracy, precision, extraction recovery and stability data for method validation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Indapamide (25ng/mL)</th>
<th>IS (15μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Accuracy (%)</td>
<td>Precision (%)</td>
</tr>
<tr>
<td>working solution Stability</td>
<td>105.5</td>
<td>4.1</td>
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<tr>
<td>stock solution Stability</td>
<td>105.9</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 2 Pharmacokinetic parameters of indapamide in 20 male volunteers after oral administration of 5 mg indapamide.

R: reference tablets; T: test tablets; mean ± SD, n=20

![Fig. 2 Mean plasma concentration-time profiles of indapamide in 20 Chinese male volunteers after oral administration of 5 mg indapamide.](image-url)
medical practice. The results of this study, obtained from samples of healthy Chinese volunteers, might serve as a reference for future controlled studies of indapamide in the Chinese population.

Acknowledgement

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Conflict of Interest Statement

The authors declare that they have no conflict of interest with respect to this paper.

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Table 3  Statistical analysis of log-transformed data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Period</th>
<th>Subject in sequence</th>
<th>90% CI (%)</th>
<th>Lower</th>
<th>Upper</th>
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<tbody>
<tr>
<td>Mosapride citrate</td>
<td>C(max)</td>
<td>0.591</td>
<td>0.6133</td>
<td>0.0001</td>
<td>100.48–106.55</td>
<td>19.11</td>
</tr>
<tr>
<td></td>
<td>AUC(0–84)</td>
<td>0.2325</td>
<td>0.5981</td>
<td>&lt;0.0001</td>
<td>99.06–105.79</td>
<td>10.53</td>
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<tr>
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<td>AUC(0–∞)</td>
<td>0.6217</td>
<td>0.1091</td>
<td>&lt;0.0001</td>
<td>96.85–105.97</td>
<td>8.1</td>
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