Relative Bioavailability of Rifampicin in Four Chinese Fixed-dose Combinations Compared with Rifampicin in Free Combinations

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Background: Decreases in the bioavailability of rifampicin (RFP) can lead to the development of drug resistance and treatment failure. Therefore, we investigated the relative bioavailability of RFP from one four-drug fixed-dose combination (FDC; formulation A) and three two-drug FDCs (formulations B, C, and D) used in China, compared with RFP in free combinations of these drugs (reference), in healthy volunteers.

Methods: Eighteen and twenty healthy Chinese male volunteers participated in two open-label, randomized two-period crossover (formulations A and C) or one three-period crossover (formulations B and D) study, respectively. The washout period between treatments was 7 days. Bioequivalence was assessed based on 90% confidence intervals, according to two one-sided t-tests. All analyses were done with DAS 3.1.5 (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

Results: Mean pharmacokinetic parameter values of RFP obtained for formulations A, B, C, and D products were 11.42 ± 3.41 μg/ml, 7.86 ± 5.78 μg/ml, 13.05 ± 6.80 μg/ml, and 16.18 ± 3.87 μg/ml, respectively, for peak plasma concentration (Cmax), 91.43 ± 30.82 μg·h⁻¹·ml⁻¹, 55.49 ± 37.58 μg·h⁻¹·ml⁻¹, 96.50 ± 47.24 μg·h⁻¹·ml⁻¹, 101.47 ± 33.07 μg·h⁻¹·ml⁻¹, respectively, for area under the concentration-time curve (AUC0–24 h).

Conclusions: Although the concentrations of RFP for formulations A, C, and D were within the reported acceptable therapeutic range, only formulation A was bioequivalent to the reference product. The three two-drug FDCs (formulations B, C and D) displayed inferior RFP bioavailability compared with the reference (Chinese Clinical Trials registration number: ChiCTR-TTRCC-12002451).

Key words: Bioequivalence; Fixed-dose Combination; Rifampicin; Tuberculosis

INTRODUCTION

Tuberculosis (TB) is still the infectious disease with the highest lethality, and China has the second highest TB burden globally. In 2013, there were notifications of 136,000 people with multidrug-resistant (MDR)-TB or rifampicin (RFP)-resistant TB (TB) who were eligible for MDR-TB treatment worldwide. The treatment of TB requires more than two drugs to prevent the emergence of resistant strains.

Fixed-dose combinations (FDCs) are combinations of two or more drugs in a single pill. The first-line anti-TB drugs, including isoniazid (INH), RFP, pyrazinamide (PZA), and ethambutol (EMB) are often the components of FDCs. According to the published research, RFP is the questionable drug in such FDCs. RFP is lipophilic, whereas the other three drugs are hydrophilic. Studies have reported differences in RFP bioavailability between products containing RFP alone and FDC products.

The use of qualified FDC formulations in the treatment of TB can increase patients’ compliance compared with free combinations of the same drugs, and reduce the risk of treatment failure and emergence of drug-resistant strains. The WHO has thus recommended the use of FDCs in the treatment of TB and has published a list of prequalified products.

The bioavailability of RFP is important in FDC. The aim of the present study was therefore to determine the bioequivalence of RFP in FDC formulations compared with RFP in free combinations in healthy Chinese male volunteers.
**Methods**

**Fixed-dose combination formulations and free combinations**

Isoniazid tablets (100 mg, batch No. 1103021), EMB tablets (250 mg, batch No. 1103921), PZA tablets (250 mg, batch No. 1103031), and RFP capsules (150 mg, batch No. 1102041) were supplied by HongQi Pharmaceutical Company (Shenyang, China) as reference drugs. The four-drug FDC formulation was a tablet containing 75 mg INH, 150 mg RFP, 275 mg EMB, and 400 mg PZA (test formulation A, batch No. 1104022). The two-drug FDC test formulations were tablet B (150 mg INH, 300 mg RFP, batch No. 110204), capsule C (75 mg INH, 150 mg RFP, batch No. 110501) and capsule D (150 mg INH, 300 mg RFP, batch No. 110401). The FDC formulations were from different manufacturers except that formulation C and D were from the same manufacture.

**In vitro evaluation**

The drug content and dissolution profiles of all formulations were determined in accordance with Chinese pharmacopeia protocols. The dissolution study was conducted at 75 r/min and 37°C using 900 ml phosphate buffer saline. Dissolved RFP was assayed at 254 nm using high-performance liquid chromatography (HPLC; Agilent 1200, USA).

**Relative bioavailability study**

**Experimental design**

Three bioavailability studies were conducted as open, two- or three-period, two- or three-sequence crossover trials. Two two-drug FDCs (B, D) containing INH and RFP were administered to a group of 18 volunteers with washout periods of 1 week between treatments. One two-drug FDC (C) containing INH and RFP was administered to a group of 20 volunteers with a washout period of 1 week. One four-drug FDC (A) containing INH, RFP, EMB, and PZA was administered to a group of 18 volunteers with a washout period of 1 week.

All volunteers underwent medical history screening, physical examination, blood pressure measurement, hemogram test, liver function test, hepatitis B virus test, chest radiography, electrocardiography, and routine urine analysis. Volunteers with a history of allergy to drugs related to the study medications or metabolic diseases, and those who had taken any drugs in the 2 weeks before the start of the study were excluded. The volunteers had no history of tobacco or alcohol use, or heart, liver, kidney or gastrointestinal disorders. The volunteers were aged between 20 and 40 years, and had a body mass index ranging from 19 to 24 kg/m².

After overnight fasting, each volunteer received either a FDC formulation or the reference product (containing a single dose of 600 mg RFP) with 200 ml water. Volunteers received a light breakfast at 4 h postdose. Smoking and the consumption of alcohol were forbidden during the study. Blood samples (3 ml each) were collected in heparinized tubes at 0, 10, 20, and 40 min and 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h postdose. Serum was separated and stored at −80°C until analysis.

**Ethics**

The study was approved by the medical ethical committee of the Beijing Chest Hospital. All volunteers gave their informed consent prior to their inclusion in the study. Chinese Clinical Trials registration number: ChiCTR-TTRRC-12002451.

**Assay method**

**High-performance liquid chromatography-tandem mass spectrometry apparatus and conditions**

An Agilent HPLC Systems apparatus (Agilent Technologies, USA) equipped with a model G1329A ALS and a column heater (G1316A) was used. The mass spectrometer (MS) was a 6410B triple quadrupole MS (Agilent Technologies, USA). An electrospray ionization source was used.

The plasma concentration of RFP was measured using a HPLC-MS/MS method developed in our laboratory. Briefly, 100 μl of plasma sample was deproteinized with 200 μl of acetonitrile. The mixture was vortexed for 10 s and centrifuged for 10 min at 12,000 g; 2 μl of the supernatant was injected into the chromatographic system. Analysis was performed using a ZORBAX SB C-18 (2.1 mm × 50 mm, 1.8 μm; Agilent). The mobile phase was a mixture of water (0.1% formic acid): Acetonitrile (40:60 v/v). A flow rate of 0.3 ml/min was employed. The multiple reaction monitoring (MRM) transition was 823.2 → 791.3 for RFP. Data were collected and processed using G3335AA-MASSHUNTER_ software-78 (Agilent Technologies, USA).

There was no interference with endogenous plasma compounds. The assay was linear over the range of 0.2–28 μg/ml (r² = 0.999). Coefficients of variation for intra- and inter-day precision were less than 5%. The limits of quantification and detection of RFP were 0.2 μg/ml and

### Table 1: Details of the bioequivalence trials of FDC formulations versus free combinations of Anti-TB drugs

| FDC code | FDC formulations | FDC dosage (mg) | Number of tablet/capsule | Number of volunteers (n) | Time (h) |
|----------|------------------|-----------------|--------------------------|-------------------------|---------|
|          | FDC             |                  |                          |                         |         |
|          | dosage (mg)      | RFP  | INH  | PZA  | EMB  |
| A        | Tablet/4 drug    | 150  | 75   | 400  | 275  | 4     | 17    | 18   | 24   |
| B        | Tablet/2 drug    | 300  | 150  |      |      | 2     | 7     | 18   | 24   |
| C        | Capsule/2 drug   | 150  | 75   |      |      | 4     | 7     | 20   | 24   |
| D        | Capsule/2 drug   | 300  | 150  |      |      | 2     | 7     | 18   | 24   |

**FDC:** Fixed-dose combination; **TB:** Tuberculosis; **RFP:** Rifampicin; **INH:** Isoniazid; **PZA:** Pyrazinamide; **EMB:** Ethambutol.
0.01 μg/ml, respectively. RFP in blood samples remained stable at −80°C over a period of 21 days.

**Pharmacokinetic and statistical analysis**

Pharmacokinetic (PK) parameters were analyzed by Drug and Statistics Software (DAS 3.1.5, Mathematical Pharmacology Professional Committee of China, Shanghai, China) using noncompartmental analysis. Peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly obtained from individual plasma concentration-time profiles. The area under the concentration-time curve to the last measurable concentration (AUC_{0–24 h}) was calculated by the linear trapezoidal method. The half-life (t_{1/2}) values were determined as 0.693/k.

Bioavailability equivalence was accepted if the 90% confidence intervals (90% CIs) calculated for the test/reference formulation mean ratios were within the ranges of 80%–125% for AUC_{0–24 h} and 70%–143% for C_{max}. The T_{max} between two treatment arms was analyzed using the Wilcoxon test, and differences were considered significant at P < 0.05.

**RESULTS**

**In vitro evaluation**

The dissolution profiles are shown in Figure 1. For the reference, the percentage of RFP dissolved after 30 min was 84.9% ± 1.32%, while for formulations A and C it was above 80% (96.2% ± 0.50% and 97.2% ± 1.64%, respectively), and for formulation B and D it was 33.7% ± 0.48% and 65.8% ± 1.05%, Formulation B and D did not meet the requirement of the dissolution test.\(^{[10]}\)

**Relative bioavailability study**

Mean concentration-time profiles of RFP for the four FDC formulations are presented in Figure 2. The PK parameters (AUC_{0–24 h}, C_{max}, T_{max}, and t_{1/2}) of RFP are given in Table 2. The primary estimates of bioequivalence are shown in Table 3. All of these PK parameters were compared for both the FDCs and free combinations. Out of four FDC formulations tested, only one, FDC A, had comparable AUC_{0–24 h} and C_{max} values of RFP to those for the corresponding free combinations and hence passed the bioequivalence test. For both the FDC and free combination, a 600-mg dose of RFP produced AUC_{0–24 h} values of 55.5–108.3 μg·h·ml⁻¹ and C_{max} values of 7.9–16.2 μg/ml.

The concentration of RFP in FDC formulation B was considerably lower compared with the free combination, at approximately 50% throughout the full sampling time interval of the reference. The 90% CIs for the C_{max} and AUC_{0–24 h} ratios were clearly outside the 70%–143% and 80%–125% ranges for the free combination, indicating the bioinequivalence of formulation B compared with the reference product.

The differences between the C_{max} of the FDC and free combination were not apparent for formulations C and D, shown in Figure 2. However, Table 2 shows that formulations C and D were not equivalent to the reference formulations. About 90% CI of AUC_{0–24 h} (76.9%–91.7%) and C_{max} (67.5%–87.6%) in formulation C were both inequivalent compared with a reference. Of the 20 volunteers, 10 had AUC_{0–24 h} values that were less than 80% of the reference and seven had C_{max} values that were <70% of the reference. The AUC_{0–24 h} of formulation D was within the range of 79–116% of the reference. Seven volunteers had AUC_{0–24 h} values that were less than 80% of the reference in formulation D (40.85, 58.49, 63.00, 76.94, 81.18, 81.63, and 84.78 μg·h·ml⁻¹), respectively. The C_{max} of formulation D passed the bioequivalence test (85.3–131.8%).

The t_{1/2} of the reference product (3.38–4.46 h) and test formulations (3.31–3.81 h) were within the interval reported by other authors.\(^{[12,13]}\)

The T_{max} for the test product was not significantly different from that of the reference product except for formulation B, for which the T_{max} was 2.78 ± 1.53 h (P < 0.05) while the reference was 1.54 ± 0.59 h.

**DISCUSSION**

Resistance to INH and RFP often results in incurable TB. The global spread of TB is further complicated by the ubiquitous appearance of MDR strains defined as those, which are resistant to at least INH and RFP.\(^{[14]}\) The effective treatment of TB requires high-quality drug products. The WHO has suggested that qualified FDCs be used for the treatment of TB.\(^{[15]}\) A clinical trial conducted at 11 sites in 9 countries supported the WHO recommendations for the use of FDCs because of the potential advantages compared with free combinations of the component drugs.\(^{[16]}\)

Fewer previous studies have been conducted in China comparing the bioequivalence of the anti-TB FDC products available there.\(^{[11]}\) Because there was no certified reference FDC product available in China at the time of this study, we used separate drugs as the reference in this bioequivalence study. Agrawal et al. suggested that the RFP bioavailability problem is more attributable to extrinsic factors such as
formulation or RFP bulk material rather than intrinsic variability of RFP absorption.\[17\] Our in vitro dissolution test showed that, although formulation C meets the dissolution requirement, it failed the bioequivalence study. The test showed that, even when RFP formulations meet dissolution requirements, the drug is not always well absorbed. Other authors have also found that the relationship between RFP dissolution properties and bioavailability is poor.\[18\]

We chose sample sizes of 18–20 volunteers and a sampling time of 24 h for our study because previous studies have shown these to be sufficient to demonstrate bioinequivalence.\[4,19,20\] In the relative bioavailability study, the RFP concentration-time profile of formulation B clearly differed from the reference, while those of the other three FDCs were similar to the reference [Figure 2]. The C_{max} and AUC_{0–24 h} of formulations A, C, and D also had no obvious difference from the reference. The RFP C_{max} value of formulations A, C, and D were above the minimum therapeutic plasma concentration (10 μg/ml), and this was comparable to the results from an Indian study, which used a dose of 600 mg.\[21\]

We examined the data of each volunteer and found substantial inter-subject variability in the data. For formulation C, half of the volunteers’ AUC_{0–24h} values were below the 80% mean

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### Table 2: Pharmacokinetic parameters of FDC formulations A, B, C and D for RFP resulted from different bioequivalence trials

| FDC code | AUC_{0–24h} (μg·h^{-1}·ml^{-1}) | C_{max} (μg/ml) | T_{max} (h) | t_{1/2} (h) |
|----------|-------------------------------|----------------|-------------|------------|
|          | FDC                           | Reference      | FDC         | Reference  | FDC         | Reference  | FDC         | Reference  |
| A        | 91.43 ± 30.82                 | 95.22 ± 29.22  | 11.42 ± 3.41| 12.38 ± 3.22| 1.69 ± 0.83 | 1.82 ± 1.21| 3.68 ± 1.25 | 4.46 ± 2.5 |
| B        | 55.49 ± 37.58                 | 106.84 ± 36.32 | 7.86 ± 5.78 | 15.38 ± 4.37| 2.78 ± 1.53 | 1.54 ± 0.59| 3.81 ± 0.96 | 3.59 ± 1.32|
| C        | 96.50 ± 47.24                 | 108.28 ± 34.38 | 13.05 ± 6.80| 15.66 ± 4.48| 1.73 ± 0.82 | 1.4 ± 0.57 | 3.31 ± 1.52 | 3.38 ± 1.77|
| D        | 101.47 ± 33.07                | 106.84 ± 36.32 | 16.18 ± 3.87| 15.38 ± 4.37| 1.68 ± 0.65 | 1.54 ± 0.59| 3.66 ± 1.28 | 3.59 ± 1.32|

All the values are given as mean ± SD. Sample size (n) is given in Table 1. FDC: Fixed-dose combination; AUC: Area under the curve; SD: Standard deviation; RFP: Rifampicin.

### Table 3: Test/reference ratios and 90% CI derived from pharmacokinetic parameters of RFP for the assessment of bioequivalence

| FDC code | Pharmacokinetic parameters | Test/reference (%) | 90% CI Bioequivalence result |
|----------|----------------------------|--------------------|-----------------------------|
| A        | AUC_{0–24h}                 | 94.7               | 89.5–100.2 Passed           |
|          | C_{max}                     | 90.9               | 81.8–100.9                  |
| B        | AUC_{0–24h}                 | 44.4               | 36.7–53.8 Failed           |
|          | C_{max}                     | 41.8               | 33.6–51.9                   |
| C        | AUC_{0–24h}                 | 84.0               | 76.9–91.7 Failed           |
|          | C_{max}                     | 76.9               | 67.5–87.6                   |
| D        | AUC_{0–24h}                 | 95.7               | 79.0–115.9 Failed          |
|          | C_{max}                     | 106.1              | 85.3–131.8                  |

Statistical evaluation was done by DAS 3.1.5. 90% CI of bioequivalence for AUC_{0–24h} is 80–125%, for C_{max} is 70–143%. FDC: Fixed-dose combination; CI: Confidence interval; AUC: Area under the curve; RFP: Rifampicin.

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Figure 2: Mean concentration-time profiles of RFP after administration of free combination and FDCs in three bioequivalence trials (Formulation a–d). Y-error bars indicate SD (for sample sizes refer to Table 1). FDC: Fixed-dose combination; RFP: Rifampicin; SD: Standard deviation.
value of the reference and one-third of the volunteers’ $C_{\text{max}}$ values were below the 70% mean value of the reference. The other volunteers had higher values of the AUC$_{0-24\,\text{h}}$ and $C_{\text{max}}$, indicating that the data were polarized. The situation was similar for formulation D, but only the AUC$_{0-24\,\text{h}}$ was slightly lower than 80% value of the reference. Based on this consideration, although sample sizes of 18 volunteers are typical for bioequivalence studies, a small sample from a single center was a limitation of this study. Furthermore, healthy male volunteers are not representative of patient populations.

Out of four FDC formulations evaluated, only one formulation was bioequivalent for RFP to free combinations of the component drugs, whereas the other three formulations were below the limits of bioequivalence. Our studies showed the importance of conducting bioequivalence studies before the distribution of FDC products within the Chinese health system.

Any reduction in the bioavailability of anti-TB drugs such as RFP could have serious consequences, including potential treatment failure and selection of drug-resistant mutants. The poor bioavailability of RFP represents a serious problem for the success of TB treatment, which must be addressed by the Chinese health authorities.

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