Serum Concentrations of Rifampin, Isoniazid, and Intestinal Absorption, Permeability in Patients with Multidrug Resistant Tuberculosis

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Abstract. This study evaluates the serum concentrations of rifampin (RMP), isoniazid (INH), and intestinal barrier function in patients with multidrug-resistant tuberculosis (MDR-TB), drug susceptible tuberculosis (DS-TB), and healthy volunteers (HC; controls). Peak serum concentrations of RMP were significantly lower in MDR-TB and DS-TB as compared with HC (odds ratio [OR] = 3.125, confidence interval [CI] [1.037–9.418] and OR = 4.025, CI [1.207–13.418], respectively). The INH peak serum concentration was not significantly different between MDR-TB versus DS-TB or DS-TB versus HC. The percent of mannitol excretion was significantly lower in the MDR-TB group compared with DS-TB (13.18 versus 16.03, analysis of covariance [ANCOVA], P = 0.0369) and compared with HC (15.18 versus 16.61, ANCOVA, P = 0.0291) the other study groups. These data suggested a lower peak serum concentration of RMP for both MDR-TB and DS-TB as compared with the HC group. The data also showed a lower intestinal area of absorption in patients with tuberculosis and even worse in MDR-TB.

INTRODUCTION

Tuberculosis continues to be an important public health problem, where one out of every three people on Earth is infected.1 Erratic, incomplete treatment, and low-peak serum concentrations of antituberculosis drugs create an important risk for multidrug-resistant tuberculosis (MDR-TB) and also extensively drug-resistant tuberculosis (XDR-TB).2,3 Recent estimates suggest that every year, nearly half a million MDR-TB cases emerge and more than 130,000 people die of MDR-TB. In certain geographic areas of the World over 20% of new tuberculosis cases are now MDR-TB, and almost 10% of MDR-TB cases are XDR-TB.4,5 In the state of Ceará, Brazil, the proportion of MDR-TB increased from 0.82% in 1994 to 1.48% in 19996 and it continues to rise (Barroso EC, unpublished data).

There are a few studies suggesting that reduced antimycobacterial drug absorption and bioavailability can delay or reduce the cure rate for tuberculosis and enhance the emergence of drug resistance.7–9 Several studies to date have shown low serum concentrations of antitubercular drugs in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) patients7–9 and others with HIV.10–12 Most previous studies of rifampin (RMP) and isoniazid (INH) have used the same daily dose, and most have not included pharmacokinetic evaluations; we postulate that these doses will not be suitable to all individuals, including patients and healthy volunteers from different racial and genetic backgrounds. In addition to these factors, intestinal barrier function and absorptive area may potentially influence antitubercular drug absorption and peak serum concentrations. In the last 10 years our group has used lactulose and mannitol absorption as markers for the paracellular and transcellular transport, respectively, in the intestinal epithelium.13,19 Only a few studies, including two from our research group, have attempted to evaluate intestinal barrier function, absorption, and serum concentrations of antimycobacterial drugs,14–18 and none to date have evaluated MDR-TB patients compared with drug-susceptible tuberculosis (DS-TB) and healthy controls (HC), without concomitant HIV infection. This study evaluates intestinal barrier function and serum concentrations of RMP and INH in outpatients with MDR-TB, DS-TB, and in HC.

MATERIALS AND METHODS

Ethical approval. The study was approved by the local and national ethical committee for research called CONEP, Conselho Nacional de Saúde, resolution Nos. 196/96, 251/97, and 292/99 from Agência Nacional Sanitária, Brasília, DF, Brazil. Consent and case report forms were submitted to the same committee and approved on April 4, 2006. All subjects were informed verbally and signed the consent form before entering the study.

Geographic location and study population. The study was performed at the Hospital de Messejana, 200 beds, from August 2006 to April 2007. The hospital is a referral center for MDR-TB located in Fortaleza city, the capital of the Ceará state in the Northeast of Brazil. The population of the state is ~8,097,276 habitants from the census done in 2005.20 The state had 41,073 new cases of tuberculosis from 1990 to 1999.5 In 2005, the total new cases of tuberculosis were 4,104 with an incidence of 50.7 per 100,000 habitants.21

Study design, inclusion, and exclusion criteria. The study was a cross-section evaluation of 127 screened individuals, including health volunteers, for serum rifampin and isoniazid peak serum concentration and intestinal barrier function. The MDR-TB was defined as a patient infected with Mycobacterium tuberculosis resistant at to least RMP and INH using the susceptibility test by the proportion method at Laboratório Central de Saúde Pública do Ceará (LACEN-CE) and using the method reported before by Canetti and others.22 All patients had MDR-TB and had been referred from throughout Ceará state to be treated using a daily dose of TB drugs and followed up at Hospital de Messejana; they were then invited
for the research. The DS-TB was defined as a patient infected with *M. tuberculosis* susceptible to all four drugs tested, RMP, INH, streptomycin, and ethambutol. They had the diagnosis of tuberculosis performed in the Hospital de Messejana. When the susceptibility test was available, the majority of patients had been referred to a health center where they were under supervised treatment, near their home, taking TB drugs daily. We contacted each patient and invited them to participate in the study after which they continued their supervised treatment, but we also followed up by appointed visits to the hospital every 2 months for 1 year after the end of the research. A healthy volunteer (HC) was considered normal control based on clinical history and laboratory studies including: total and differential blood cell counts, blood biochemistry, liver and kidney function tests, total and serum proteins, and anti-HIV test. They were selected among health care workers of the Hospital or relatives and friends of the research group. All groups of patients and healthy volunteers were matched by age and sex with MDR-TB group.

The inclusion criteria were 1) meet the case definition or be a matched, healthy volunteer; 2) greater than 15 years of age; and 3) agree to participate in the study and sign the consent form. The exclusion criteria were 1) no isolation of *M. tuberculosis* in MDR-TB and DS-TB patients; 2) isolation of nontuberculous mycobacteria; 3) clinical history of allergy to RMP or INH; or 4) diagnosed with cancer, gastrointestinal diseases, liver or kidney dysfunction, or other systemic severe diseases.

**Social-demographic, clinical, and nutritional evaluation.** A case report form was developed and used to collect social-demographic and clinical data at the enrollment of each patient or control after signing the consent form. The period between the first TB treatment and the initiation of the study was called PITS, and the period between the current treatment and the initiation of the study, PCTS. The history of alcohol ingestion was taken based on a previous report by O’Connor and Schottenfeldt. The smoking history was based on the classification by Fahn and others.

Weight (kg) and height (m) were taken on the day of study enrollment using a calibrated scale with an accuracy of 100 g and a height measuring stick with an accuracy of 1 mm. The body mass index (BMI; kg/m²) was assessed using the World Health Organization (WHO) reference values.

**Blood biochemistry.** Participants were asked to undergo an early morning fast. Blood samples (5–10 mL) were collected at 2 and 6 hours after antitubercular drug administration (RMP + INH). The following blood biochemistry substances were assayed: 1) glucose; 2) urea; 3) creatinine; 4) amino-transferases (aspartate-AST and alanine-ALT); 5) total, direct, and indirect bilirubin; 6) total and fractional protein; and 7) HIV test. All blood biochemistry measurements were done using a quality control protocol for Good Laboratory Practice, an automatic system at the Chemistry Clinical Laboratory, Messejana’s Hospital, Secretary of Health, Ceará, Brazil.

**Intestinal permeability test.** Briefly, a solution containing lactulose (250 mg/mL; Lactulona, Luitold Produtos Farmacêuticos Ltda, S. Paulo, SP, Brazil) and mannitol (50 mg/mL; Mannitol, Henri Farma Produtos Químicos e Farmacêuticos Ltda, S. Paulo, SP, Brazil) in 20 mL sterilized and distilled water was used for the intestinal permeability test. This solution was prepared at the Clinical Research Unit and Institute of Biomedicine, School of Medicine, UFC. All individuals were asked to fast after midnight and their bladder emptied at 08:00 hours (time 1; Figure 1), immediately before the lactulose: mannitol test solution was administered orally. Urine samples were collected for the next 5 hours and mixed with one drop (50 μL) of clorhexidine (40 mg/mL; Sigma Chemical Co., St. Louis, MO) per 50 mL of urine. The total urine collected for each individual was measured (mL) and an aliquot of 1.5 mL was preserved and stored at −80°C until the amount of lactulose and mannitol could be measured by high performance liquid chromatography with pulsed amperometric detection (HPLC-PAD). This method and the quality control HPLC-PAD method was based on previous work published elsewhere.

**Rifampin and isoniazid in vitro bioequivalence and serum measurements.** All patients and healthy volunteers received the same lot no. 05070517 (valid until July 2007 from Laboratório Farmacêutico do Estado de Pernambuco—LAFEPE, Recife, PE, Brazil) of fixed combined pill doses for RMP plus INH, 150 and 100 mg, respectively. The quality control reported from Agência Nacional de Vigilância Sanitária—ANVISA, Brasília, DF, Brazil, showed 96% and 97% of the original dose for RMP and INH, respectively.

All patients and healthy volunteers were asked to fast after midnight and those taking medication to treat MDR-TB, ingested their pills until 18:00 hours the day before from the collection of serum samples. The patients with DS-TB were asked not to take their medication at home, on the day of the study. The doses of RMP and INH for each patient were calculated by kilogram of body weight, following the guidelines from Program Nacional da Tuberculose, Ministério da Saúde, Brasília, DF, Brazil, as published elsewhere (600 mg of RMP for weight > 45 kg and 450 mg for 35 < weight ≤ 45 kg; 400 mg of INH for weight > 45 kg and 300 mg for 35 < weight ≤ 45 kg). All doses were taken with 150 mL of water and under direct observation by one of the investigator participants in the study protocol at 07:00 hours (time 0; Figure 1). Blood samples (8 mL) were collected from all participants in the study using tubes with EDTA, two (09:00 hours – time 2; Figure 1) and six (13:00 hours – time 6; Figure 1) hours after the ingestion of the drugs. Serum was separated by centrifugation and frozen at −80°C within 1 hour after collection. Rifampin and isoniazid serum concentrations were assayed at Dr. Pelouquin’s laboratory using a validated high-performance liquid chromatography. All drugs had a mean dose within 107–121% of the stated amount referenced in the pills. Peak serum concentrations and normal serum range of these drugs were validated using the 2-hour blood samples. Normal serum concentrations were 8–24 μg/mL and 3–6 μg/mL for RMP and INH, respectively. Low and very low peak serum concentrations for RMP were < 8 and < 4 μg/mL, respectively. For INH, it was < 3 and < 2 μg/mL, respectively, for low and very low peak serum concentrations.

**Sample size calculation and statistical analysis.** Sample size was calculated using both lactulose: mannitol ratio or antitubercular drug serum concentrations. On the basis of our previous studies in Fortaleza, we would expect a 30% increase in lactulose: mannitol ratio or a 30% reduction in serum drug concentrations in cases compared with controls. We estimated a sample size of at least 23 for each group to detect a significant difference between these groups, using a power of 90% and a two-sided significant level of 5%. Assuming we could have a 10% loss, then an estimate of at least 26 subjects in each group was calculated.
All the data were entered twice by two independent persons and validated using the Excel software version 4.0 (Microsoft Co., Seattle, WA). The statistical analyses were done using the Statistical Package for Social Sciences version 11.5 (SPSS Inc., Chicago, IL). The normality and variance of quantitative variables were tested using the Shapiro-Wilk and Levene tests, respectively. Any parameter not following the normal distribution were math transformed or analyzed using non-parametric tests, such as Kruskal Wallis, Mann-Whitney tests, $\chi^2$ tests, or Fisher exact tests. For multiple comparisons between groups, the Kruskal Wallis sub-hypothesis test was used. Normally distributed, continuous variables were analyzed using the Student’s $t$ test. Covariance analysis (ANCOVA) was used to correct the influence of several factors such as BMI, alcohol, and smoke dependence when compared with intestinal permeability parameters or serum drug concentrations. The figures were done using GraphicPad Prism software 3.0 (GraphPad Software, San Diego, CA). The $P$ values of 0.05 and less were considered a statistically significant difference.

RESULTS

A flow diagram of all eligible subjects is shown in Figure 1. The total individuals in each group of the study were 32, 28, and 30 for TBMR, DS-TB, and HC, respectively (Figure 1).

The characteristics of the individuals selected to enter the study protocol are summarized in Table 1. The median/range of age for all individuals was 42.5/17–69 (years). Forty-five (50%; 45/90) were male. There were no significant differences between study groups for the following parameters analyzed 1) age; 2) sex; 3) geographic location of the individuals; 4) associated pathology such as diabetes mellitus, other pulmonary diseases, HIV/AIDS infection, psychiatry disorders, and intestinal parasites; 5) severity of pulmonary lesions; and 6) detection of the $M. tuberculosis$ in the initiation of the study.
protocol. All the individuals, except one (DS-TB patient who refused to do the test), were negative for the anti-HIV test. The blood biochemistry substances measured, including glucose, liver and renal functional tests, total proteins and fractions were within normal range for all groups.

The lactulose: mannitol ratio was similar among the three groups ($P = 0.4747$). The paracellular transport of lactulose was significantly reduced in MDR-TB patients (median/range, 0.25%/0.0–0.937) compared with HC (0.408%/0.0–1.902; $P < 0.05$) and the mannitol transcellular transport (median/range, 12.9%/13.0–38.4) was significantly lower in MDR-TB (5.37%/0.21–12.60) compared with HC controls ($P = 0.0027$) (Table 2B). Further analysis, adjusting the peak serum concentration for RMP in MDR-TB and HC groups that were using the drug only for the study, showed consistent lower peak serum concentration of this drug for MDR-TB and DS-TB groups compared with HC (Figure 2B). Considering the normal range of serum concentration of RMP (8–24 μg/mL), the data showed a significantly higher proportion of patients below 8 μg/mL in the MDR-TB (78%; 23/32) compared with HC (50%; 15/30), $P < 0.05$ and in DS-TB (82%; 23/28) compared with HC, $P < 0.05$ (Table 3). This analysis is also consistent if below the very low serum concentration of RMP (< 4 μg/mL) is considered (Table 3).

By linear correlation analysis, RCMAX was found to be positively correlated with dose per kilogram of body weight only for the HC group ($r = 0.529, P = 0.0027$) (Table 4).

The peak serum concentration for isoniazid (ICMAX) was not significantly different comparing MDR-TB (3.89/1.01–

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**Table 1**

The characteristics at baseline of the multidrug-resistant tuberculosis (MDR-TB), drug-susceptible tuberculosis (DS-TB), and healthy controls (HC) groups in Ceará-Brazil, from August 2006 to April 2007

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>MDR-TB N = 32</th>
<th>$P^*$ values</th>
<th>DS-TB N = 28</th>
<th>$P^*$ values</th>
<th>HC N = 30</th>
<th>$P^*$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years$§$</td>
<td>41.5 (17.0–61.0)</td>
<td>NS§§</td>
<td>36.5 (21.0–69.0)</td>
<td>NS§§</td>
<td>44.0 (19.0–58.0)</td>
<td>NS§§</td>
</tr>
<tr>
<td>Male n (%)</td>
<td>15 (46.9)</td>
<td>NS¶¶</td>
<td>16 (57.1)</td>
<td>NS¶¶</td>
<td>14 (46.7)</td>
<td>NS¶¶</td>
</tr>
<tr>
<td>Alcoolismo Grave n (%)</td>
<td>10 (31.3)</td>
<td>NS¶¶</td>
<td>12 (42.9)</td>
<td>S¶¶</td>
<td>2 (6.7)</td>
<td>S¶¶</td>
</tr>
<tr>
<td>Smoker (pack years)$§$</td>
<td>1.6 (0.0–153.0)</td>
<td>NS§$</td>
<td>9.5 (0.0–73.5)</td>
<td>S§§</td>
<td>0.0 (0.0–30.0)</td>
<td>S§§</td>
</tr>
<tr>
<td>Drug use n (%)</td>
<td>2 (6.3)</td>
<td>NS§§</td>
<td>10 (35.7)</td>
<td>S§§</td>
<td>1 (3.3)</td>
<td>S§§</td>
</tr>
<tr>
<td>BMI$¶$ (kg/m²)$§$</td>
<td>19.9 (13.0–38.4)</td>
<td>NS§§</td>
<td>20.3 (15.7–26.3)</td>
<td>S§§</td>
<td>24.8 (19.2–38.6)</td>
<td>S§§</td>
</tr>
<tr>
<td>Weight loss (%)$§$</td>
<td>–5.3 (–39.2–12.5)</td>
<td>NS§§</td>
<td>–4.9 (–19.0–7.8)</td>
<td>S§§</td>
<td>0.0 (–2.8–9.2)</td>
<td>S§§</td>
</tr>
<tr>
<td>Total proteins (mg/dL)$§$</td>
<td>7.3 (6.3–8.5)</td>
<td>NS§§</td>
<td>7.5 (5.7–8.6)</td>
<td>S§§</td>
<td>7.2 (6.5–7.9)</td>
<td>S§§</td>
</tr>
<tr>
<td>Albumin (mg/dL)**</td>
<td>4.17 ± 0.3</td>
<td>NS</td>
<td></td>
<td></td>
<td>4.14 ± 0.47</td>
<td>S</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)$§$</td>
<td>12.9 (8.3–16.1)</td>
<td>NS</td>
<td></td>
<td></td>
<td>12.3 (7.5–17.1)</td>
<td>S</td>
</tr>
<tr>
<td>Previous TB therapy (number)$§$</td>
<td>3.0 (0.0–8.0)</td>
<td>S***</td>
<td>0.0 (0.0–2.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PITS$††$ (years)$§$</td>
<td>4.5 (0.3–38.6)</td>
<td>S***</td>
<td>0.2 (0.0–15.0)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PCT$¶$ (weeks)**</td>
<td>36.5 ± 22.0</td>
<td>S†††</td>
<td>6.4 ± 4.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RMP dose/body weight (mg/kg)**</td>
<td>10.41 ± 1.67</td>
<td>NS§§</td>
<td>10.72 ± 1.14</td>
<td>S§§</td>
<td>9.05 ± 1.43</td>
<td>S§§</td>
</tr>
<tr>
<td>INH dose/body weight (mg/kg)**</td>
<td>6.94 ± 1.12</td>
<td>NS§§</td>
<td>7.15 ± 0.76</td>
<td>S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MDR-TB versus DS-TB
† MDR-TB versus HC.
§ Median (range).
|| Body Mass Index = weight/height square.
| Current weight/former weight.
** Mean ± standard deviation.
†† Period between first TB treatment and the initiation of the study.
††† Period between current treatment and the initiation of the study.
¶¶ Odds ratio.
|| Minimum squares multiple comparisons.
*** Mann-Whitney test.
# Student’s unpaired $t$ test.
||| Tamhane multiple comparisons.

P $< 0.05$ was considered a statistically significant difference ($S$).

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**Table 2**

Covariance analysis for the percentage of mannitol excretion including the following factors: weight loss, body mass index, albumin, hemoglobin, and alcoholism + smoker in multidrug-resistant tuberculosis (MDR-TB), drug-susceptible tuberculosis (DS-TB), and healthy controls (HC).

<table>
<thead>
<tr>
<th>Percentage of mannitol excretion</th>
<th>MDR-TB</th>
<th>$P^*$</th>
<th>DS-TB</th>
<th>$P^*$</th>
<th>HC</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean standard deviation</td>
<td>13.18 ± 0.965</td>
<td><strong>0.037†</strong></td>
<td>16.03 ± 1.077</td>
<td>0.7307‡</td>
<td>16.61 ± 1.157</td>
<td><strong>0.029</strong></td>
</tr>
</tbody>
</table>

* ANCOVA = analysis of covariance.
† MDR-TB versus DS-TB.
‡ MDR-TB versus HC.
§ DS-TB versus HC.
4.72 μg/mL) versus DS-TB (3.26/0.77–4.09 μg/mL) or DS-TB versus HC, but the peak concentration was higher on MDR-TB compared with HC (2.85/0.65–3.67 μg/mL; \( P < 0.01 \)) (Figure 2C).

The proportion of patients with a low (< 3 μg/mL) serum concentration of INH were also significantly reduced in the MDR-TB compared with HC (Table 3).

By linear correlation analysis, IC\text{max} was found to be positively correlated to dose per kilogram of body weight for all groups: HC (\( r = 0.378, P = 0.0396 \)), DS-TB (\( r = 0.615, P = 0.0005 \)), and MDR-TB (\( r = 0.388, P = 0.0329 \)) (Table 4).

One year after the last patient entered the study, we assessed the treatment response in the study population. Seventeen of

**Figure 2.** Scatter plot of the maximum serum concentrations of antimycobacterial drugs within the normal range by multidrug-resistant tuberculosis (MDR-TB), drug-susceptible tuberculosis (DS-TB), and healthy controls (HC) groups in Ceará-Brazil, from August 2006 to April 2007. Maximum serum concentration was defined as the highest serum concentration independent of serum sample time collected. Panel (A) shows the scatter plot of the rifampin (weight-adjusted dose, 450 or 600 mg) maximum serum concentration; (B) shows the scatter plot of the rifampin (weight-adjusted dose, 450 or 600 mg) maximum serum concentration with MDR-TB and HC groups adjusted results according to Reference 30; and (C) isoniazid (weight adjusted dose, 300 or 400 mg) maximum serum concentration.

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**Table 3**

Proportions of the volunteers in multidrug-resistant tuberculosis (MDR-TB), drug-susceptible tuberculosis (DS-TB), and healthy controls (HC) groups below the lower therapeutic limits for rifampin and isoniazid

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Reference range</th>
<th>MDR-TB</th>
<th>( P^* ) values</th>
<th>DS-TB</th>
<th>( P^* ) values</th>
<th>HC</th>
<th>( P^* ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>100.0</td>
<td>28</td>
<td>100.0</td>
<td>30</td>
<td>100.0</td>
</tr>
<tr>
<td>2hRC§</td>
<td>&lt; 8 μg/mL</td>
<td>25</td>
<td>78.1</td>
<td>23</td>
<td>82.1</td>
<td>16</td>
<td>53.3</td>
</tr>
<tr>
<td>6h RC¶</td>
<td>&lt; 8 μg/mL</td>
<td>31</td>
<td>96.9</td>
<td>28</td>
<td>100.0</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td>RC\text{max}</td>
<td>&lt; 8 μg/mL</td>
<td>25</td>
<td>78.1</td>
<td>23</td>
<td>82.1</td>
<td>15</td>
<td>50.0</td>
</tr>
<tr>
<td>RC\text{max}</td>
<td>&lt; 4 μg/mL</td>
<td>11</td>
<td>34.4</td>
<td>19</td>
<td>67.9</td>
<td>3</td>
<td>10.0</td>
</tr>
<tr>
<td>2hIC\text{**}</td>
<td>&lt; 3 μg/mL</td>
<td>9</td>
<td>28.1</td>
<td>11</td>
<td>39.3</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>6hIC\text{††}</td>
<td>&lt; 3 μg/mL</td>
<td>27</td>
<td>84.4</td>
<td>28</td>
<td>100.0</td>
<td>30</td>
<td>100.0</td>
</tr>
<tr>
<td>IC\text{max}\text{‡‡}</td>
<td>&lt; 3 μg/mL</td>
<td>6</td>
<td>18.8</td>
<td>11</td>
<td>39.3</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>IC\text{max}\text{‡‡}</td>
<td>&lt; 2 μg/mL</td>
<td>3</td>
<td>9.4</td>
<td>5</td>
<td>17.9</td>
<td>8</td>
<td>26.7</td>
</tr>
</tbody>
</table>

* MDR-TB vs. DS-TB.
† DS-TB vs. HC.
‡ MDR-TB vs. HC.
§ Two-hour serum rifampicin concentration.
¶ Six-hour rifampicin concentration.
∥ Rifampicin maximum serum concentrations defined as the highest measurement independent of sample time collected.
** Two-hour serum isoniazid concentration.
†† Six-hour serum isoniazid concentration.
‡‡ Rifampicin maximum serum concentrations defined as the highest measurement independent of sample time collected.
§§ Fisher Exact test
S = significant \( P \) value; NS = not significant.
32 MDR-TB patients (53%) had cured, 38% (12/32) had treatment failure, and 9% (3/32) died. Twenty-four of 28 patients with DS-TB (86%) had been cured, 7% (2/28) had discontinued their treatment, and 7% (2/28) were lost to follow-up. There was a significant difference in cure rates between MDR-TB and DS-TB patients, 53% versus 86% (Fischer exact test, \( P = 0.0114 \)). All healthy volunteers continued in good health after 1 year of follow-up.

**DISCUSSION**

Several factors have been studied and they are potentially associated with development of drug resistance in the treatment of tuberculosis.\(^ {30,31} \) Among these factors cited are non-adherence to treatment, factors related to patient or the interaction between health team assistance and the patient, extreme poverty, severe disease, alcoholism, smoker, inadequate or incorrect doses, drug intolerance, and failure to deliver anti-tuberculosis drugs.\(^ {30,31} \) Two factors that remain poorly understood include intestinal barrier and absorptive function and bioavailability as indirectly measured by the intestinal lactulose:mannitol ratio and peak serum drug concentrations, respectively.\(^ {19,26,29,32} \)

This study evaluates the intestinal barrier function and peak serum rifampin and isoniazid concentrations and reviews findings specific for patients with multidrug-resistant tuberculosis compared with those with drug susceptible tuberculosis and healthy volunteers. Studies done by our group and others have shown that patients with tuberculosis or co-infected with HIV/AIDS have a decreased intestinal absorptive area that may relate to low serum concentrations of anti-tuberculosis drugs.\(^ {5,13,16} \) None to date have evaluated patients with MDR-TB as done here. It is possible that this condition is even worse and might be related to a decreased drug absorption and consequently low peak serum drug concentration. The data in this study show interesting results related to these two possibilities. First, in a multivariate analysis we find that the excretion rate of mannitol, a good marker for intestinal area of absorption, was significantly lower in the MDR-TB group than in the DS-TB and HC (Table 2). Second, barrier disruption may alter drug uptake. In a previous study\(^ 8 \) and unpublished work (Façanha MC, unpublished data), we found that DS-TB had significantly lower lactulose excretion ratios than HC patients, indicating reduced intercellular or tight junction permeability.\(^ {9,33} \) The data shown here demonstrated that this effect was influenced by weight loss, BMI, albumin, hemoglobin, and the association of alcohol plus smoking (the significant decrease in the percentage of lactulose excretion in MDR-TB versus HC disappeared after multivariate analysis included these factors) instead of the influence of tuberculosis itself.\(^ {34–37} \) This last effect may have influenced in part the lactulose:mannitol ratio in such a way that it did not show a significant difference between all groups studied. This is also consistent with another study.\(^ 8 \) Because several factors noted previously could influence intestinal barrier function and peak serum drug concentrations, a correlation of intestinal permeability parameters with serum drug concentrations was not seen in these data. It is reasonable to think that the low serum concentration of RMP and decreased intestinal area of absorption in the MDR-TB group might influence the efficacy of the therapeutic regimen for tuberculosis. Further well-designed cohort studies will be needed to understand the causality of these factors associated with antituberculosis drug resistance and treatment failure.

There are several studies that were conducted on the pharmacokinetics of first choice drugs for tuberculosis, but almost none in the drug resistant tuberculosis.\(^ {8–10,27} \) This study focuses on the peak serum rifampin and isoniazid concentrations on MDR-TB compared with DS-TB and healthy volunteers matched by age and sex. Overall, the literature has reported reduced serum concentrations of RMP and INH in patients with tuberculosis (compared with uninfected controls) with or without HIV/AIDS.\(^ {8–10,29} \) In this report, we again note low serum concentrations of RMP and INH in patients with tuberculosis, DS-tuberculosis, and healthy controls. New in this study is the significant decrease in serum rifampin concentration in MDR-TB and DS-TB compared with HC after adjusting for rifampin liver enzyme inducing drug metabolism in patients taking a daily dose.\(^ {29} \) Although the study had a limitation on the estimation of serum rifampin concentration for MDR-TB and HC, this is an important finding because MDR-TB patients are already at increased risk for having multidrug resistance and have a high risk for treatment failure and consequently more deaths as seen in the treatment assessment of these patients after 1 year of follow-up.

A recent study showed that increased doses of RMP (600 mg versus 450 mg) significantly reduced the proportion of patients with low serum rifampin concentrations without significantly increasing the severe side effects of this drug.\(^ {17} \) This dose was used in some of the patients in the MDR-TB group with body weight over 45 kilograms, as per the guidelines mentioned in the method. In their study, they used different formulation for RMP and INH, whereas in this report the combined formulation of both drugs was used, although the in vitro bioequivalence was documented twice by independent laboratories. Some authors have observed that INH accelerates degradation of RMP in the acidic conditions of the stomach when administered in fixed dose combination.\(^ {36–40} \) So, it is important to understand this low serum rifampin concentration in this study group and further double-blind studies with different doses of RMP will be critical to define an optimal dose of RMP before suggesting any change on the combination drugs treatment of tuberculosis.

The proportion of patients in the MDR-TB and DS-TB groups with low serum isoniazid concentrations is also high in this study and was consistent with data reported by others for DS-TB patients.\(^ {8–10} \) The significantly higher proportion of low serum isoniazid concentrations, found in this study, in the

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**Table 4**

<table>
<thead>
<tr>
<th>Group</th>
<th>RMP dose (mg/kg)</th>
<th>INH dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R^* )</td>
<td>( P )</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>RCmax‡ 0.108</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>ICmax‡ 0.384</td>
<td>S</td>
</tr>
<tr>
<td>DS-TB</td>
<td>RCmax‡ 0.058</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>ICmax‡ 0.596</td>
<td>S</td>
</tr>
<tr>
<td>HC</td>
<td>RCmax‡ 0.529</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>ICmax‡ 0.382</td>
<td>S</td>
</tr>
</tbody>
</table>

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*Pearson’s linear correlation.
† Isoniazid maximum serum concentration independent of sample time collected.
‡ Isoniazid maximum serum concentration independent of sample time collected.
S = significant \( P \) value; NS = not significant.
HC group compared with MDR-TB may be explained by the significant positive correlation between dose per kilogram of body weight and ICmax found here and in others studies.\textsuperscript{15,22}

In conclusion, we found an important proportion of individuals with low serum rifampin and isoniazid in all three groups studied. Serum rifampin concentrations were significantly lower in the MDR-TB and DS-TB compared with HC. The MDR-TB had a significantly reduced intestinal absorptive area compared with DS-TB and HC. In addition, alcoholism plus smoking, weight loss, BMI, albumin, and hemoglobin were significantly associated with reduced intestinal permeability via paracellular intestinal transport, and this was independent of infection with tuberculosis. These data warrant further studies investigating the efficacy of current guidelines for peak serum concentrations and dosing of RMP and INH for combined therapy of tuberculosis in different groups of patients around the world.

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