Intestinal Barrier Function and Serum Concentrations of Rifampin, Isoniazid and Pyrazinamide in Patients with Pulmonary Tuberculosis

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Intestinal barrier function and serum concentrations of rifampin, isoniazid and pyrazinamide were studied in healthy controls and patients with active pulmonary tuberculosis. A case-control study of 29 controls and 30 cases attending at the Health Center, July, 2004 to December, 2005 was conducted. The body mass index was significantly reduced in cases compared to controls ($p < 0.001$). The intestinal paracellular transport of lactulose was significantly ($p = 0.019$) reduced in cases compared to controls. The transcellular transport of mannitol and the lactulose:mannitol ratio were not significantly ($p = 0.0698$) reduced in cases compared to controls. Low serum concentrations of rifampin, isoniazid and pyrazinamide were observed in 81% (48/59), 92% (54/59) and 28% (12/59), respectively, in all individuals. The results demonstrated a marked decrease on intestinal paracellular transport in patients with active pulmonary tuberculosis and reduced serum concentrations of rifampin and isoniazid in both groups.

Key-Words: Tuberculosis, bioavailability of antimycobacterial drugs, intestinal barrier function, rifampin, isoniazid, and pyrazinamide.

Tuberculosis continues to be an important public health problem, especially in developing countries where it is associated with poverty, hunger, overcrowding, and inadequate public health systems [1]. The incidence and mortality rates of tuberculosis worldwide (2004) are 140 and 27 per 100,000 inhabitants, respectively [1]. In Brazil (2005), the incidence and mortality rates for tuberculosis are 60 and 8.1 per 100,000 inhabitants [2]. In Fortaleza (2003), the capital city of Ceará state, Northeastern Brazil, the incidence is 91 and mortality 5.6 per 100,000 inhabitants [3]. In contrast, in a developed country like United States of America (2005), the incidence and mortality rates are much lower, 5 and less than one per 100,000 inhabitants, respectively [1]. The difference in mortality between developed and non-developed countries and regions is smaller than the incidence due to the high rate of cure with combination treatment using available antimycobacterial drugs. When *Mycobacterium tuberculosis* shows resistance to first line antimycobacterial drugs, it becomes a significant problem, because the second-line drugs produce lower cure rates for this disease. In addition, second line combination drugs will prolong treatment duration, increase adverse events, reduce adherence and increase cost per treatment [4].

The worldwide prevalence of multidrug resistant tuberculosis (MDR-TB) from 1999 to 2002 is estimated at 1.7% for general patients, 1.1% in new cases and 7% for previously treated cases [5]. While these prevalence rates are low, the prevalence rate of MDR-TB is rapidly increasing worldwide [6]. In addition, there is now a more aggressive disease caused by isolates with extended resistance (XDR) to rifampin, isoniazid and other antimycobacterial drugs including fluoroquinolones and aminoglycosides [6]. In the state of Ceará, Brazil, the prevalence rate of MDR-TB increased from 0.82% in 1994 to 1.48% in 1999 [7].

Some studies conclude that the role of low antituberculosis drugs concentration remains uncertain [18,20] still, others suggest that reduced antimycobacterial drug absorption and bioavailability can delay or reduce the cure rate for tuberculosis and enhance the emergence of drug resistance [8-11]. Several studies to date have shown low serum concentrations of antimycobacterial drugs in HIV/AIDS patients [11-17], but only a few on tuberculosis patients without HIV [10,11,18-20]. Since the standard dose of an antimycobacterial drug is mostly based on trials in healthy volunteers, we postulate that this dose will not be suitable to all individuals, including patients and healthy volunteers from different racial and genetic backgrounds.

Few studies have attempted to evaluate intestinal barrier function, and absorption and serum concentrations of antimycobacterial drugs [10,13,17], and none to date have evaluated healthy controls compared to patients with tuberculosis, but without concomitant HIV infection. The present study evaluates intestinal barrier function and serum concentrations of rifampin, isoniazid and pyrazinamide in healthy volunteers and outpatients with active pulmonary tuberculosis.

Materials and Methods

Ethical Approval

The study protocol was approved (25Mar04) by the local Human Research Ethics Committee of the Federal University of Ceará and complied with the Declaration of Helsinki (1965).

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The local ethical committee is regulated by National resolution numbers 196/96, 251/97 and 292/99 from the Agência Nacional de Vigilância Sanitária, Brasília, DF, Brazil. The approved consent form was read and signed by each individual before study enrollment.

Study Design, Population and Geographic Area

This case-control study was conducted between July 2004 and December 2005. Thirty outpatients with active pulmonary tuberculosis (cases) attending the Carlos Ribeiro Health Center were matched by sex and date of birth with twenty nine healthy volunteers (controls). The Health Center is located in an urban area of Fortaleza, estimated population of approximately 2.4 million (2005), capital of the state of Ceará in the Northeast of Brazil. The Health Center treated 143, 114 and 105 new cases of tuberculosis per year from 2003-2004. The prevalence of HIV in the population with tuberculosis was very low (0.44%) as previously reported [21].

The following inclusion criteria were used for cases: (a) be a resident of Fortaleza and enrolled in the directly observed treatment (DOT) program for tuberculosis at Health Center Carlos Ribeiro; (b) more than 18 years old; (c) have at least two positive direct microscopy for acid-fast bacteria in sputum samples; (d) signed the consent form; (e) intake of antimycrobacterial drugs (rifampin, isoniazid and pyrazinamide) for less than two months. The exclusion criteria were as follows: (a) less than 18 years old; (b) history of diabetes mellitus, gastrointestinal disease, HIV/AIDS, or any other medical illness that could interfere with the pharmacokinetics of antimycobacterial drugs; (c) currently pregnant.

Social-Demographic Data and Nutritional Evaluation

Social and demographic information was collected using a questionnaire form. Alcohol dependence was evaluated using the CAGE (*cut-annoyed-guilty-eye*) questionnaire as previously described [22,23]. Weight (kg) and height (m) were taken on the day of study enrollment using a calibrated weight meter with an accuracy of 100 g and a meter with an accuracy of 1 mm. The body mass index (BMI; kg/m^2) was assessed using the World Health Organization reference values [24].

Blood Biochemistry

Participants were asked to undergo an early morning fast. Blood samples (5-10 mL) were collected at 2 and 6 hours after antimycobacterial drug administration (rifampin, isoniazid and pyrazinamide). The following blood biochemistry substances were assessed using the first sample collection: (a) glucose; (b) urea; (c) creatinine; (d) aminotransferases (aspartate-AST and alanine-ALT); (e) total, direct and indirect bilirubin; and (f) total and fractional protein. All blood biochemistry measurements were done using a quality control protocol for Good Laboratory Practice, an automatic system at the Chemistry Clinical Laboratory, University Hospital, Federal University of Ceará (UFC).

Intestinal Permeability Test

An intestinal permeability test was done using a standard solution containing lactulose (250 mg/mL; Lactulona®, Luitpold Produtos Farmacêuticos Ltda, S. Paulo, SP, Brazil) and mannitol (50 mg/mL; Manitol, Henri Farma Produtos Químicos e Farmacêuticos Ltda, S. Paulo, SP, Brazil) in 20 mL sterilized and destilled water. The standard solution was prepared at the Biotechnology Laboratory, Clinical Research Unit & Institute of Biomedicine, School of Medicine, UFC. All individuals were fasting for at least 3 hours before the lactulose:mannitol test solution was administered orally. Urine samples were collected for the next five 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). The quality control HPLC-PAD method was based on previous work published elsewhere [25].

Antimycobacterial Drugs *in vitro* Bioequivalence and Serum Measurements

Capsules with fixed dose of rifampin (300 mg) and isoniazid (200 mg) were used for serum concentration assessments of these drugs (Lote 0638, Laboratório Far-Manguinhos, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil). Pyrazinamide tablet (500 mg) was also used for serum concentration assessment of this drug (Lote 0313, Laboratório Química do Estado de Goiás S.A. – IQUEGO, GO, Brazil). These doses are consistent with antimycobacterial drug doses usually used in the treatment of tuberculosis in the Northeast of Brazil. External quality control for drug content in the dosage forms for rifampin, isoniazid and pyrazinamide was done at the National Jewish Medical and Research Center in Denver, Colorado, USA, using a validated high-performance liquid chromatography (rifampin and isoniazid) and gas chromatography with mass spectrometry (pyrazinamide) [26-28]. All drugs had a mean dose within 107% to 121% of the stated amount.

All drugs were administered orally thirty minutes before the oral ingestion of the lactulose:mannitol test solution. Blood samples were collected in tubes at 2 and 6 hours after oral administered antimycobacterial drugs. Serum was separated by centrifugation and frozen -80 °C within one hour after collection. Antimycobacterial drugs (rifampin, isoniazid and pyrazinamide) serum concentrations also were assayed at National Jewish as described above.

All patients and health controls took 600 mg of rifampin, 400 mg of isoniazid and 2 g of pyrazinamide. The usual range of normal serum concentrations of rifampin (600 mg) was 8-24 μg/mL, isoniazid (400 mg) 4-6 μg/mL and pyrazinamide (2 g) 30-60 μg/mL [11,29]. Serum concentration was considered low when rifampin was <8 μg/mL, isoniazid <4 μg/mL and pyrazinamide <30 μg/mL [11,29]. Serum concentration was

considered very low when rifampin was <4 µg/mL, isoniazid <2 µg/mL and pyrazinamide <15 µg/mL [11,29].

Sample Size Calculation and Statistical Analysis

Sample size was calculated using both lactulose:mannitol ratio or antimycobacterial drugs serum concentrations. Based on previous studies in Fortaleza [10,25], we would expect a 30% reduction in lactulose:mannitol ratio or drugs serum concentrations in cases compared to 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 estimated of at least 26 subjects in each group was calculated.

The data collected were entered twice by two independent persons in a computer database and further validated using Excel software version 4.0 (Microsoft Co., Seattle, WA). The normality and variance of quantitative variables were tested using the Shapiro-Wilk and Levene tests, respectively. Any parameters not following the normal distribution were analyzed using Mann-Whitney tests, Chi-square tests or Fisher exact tests. Normally distributed, continuous variables were analyzed using Student's t tests. Covariance analysis (ANCOVA) was used to correct the influence of BMI and alcohol dependence when compared lactulose:mannitol ratio or drugs serum concentrations between groups. The linear Pearson's correlation and multiple regression analysis were used for these parameters after adjusting for BMI and alcohol dependence. All statistical analyses were performed using the Statistical Package for Social Sciences version 11.5 (SPSS Inc. Chicago, IL). The figures were done with GraphPad Prism software version 3.0 (GraphPad Software, San Diego, CA). The alpha value of 0.05 or less was accepted as identifiying a statistically significant difference.

Results

A flow diagram of all eligible subjects is shown in Figure 1. A total of 94 individuals (63 cases and 31 controls) were selected to enter the study protocol. Fifty nine (63%; 59/94) signed the consent form and entered the study protocol. Thirty five (37%; 35/94) did not enter the study protocol for the following reasons: (a) 9 did not show up when invited to participate in the study protocol (7 cases and 2 controls); (b) 6 cases had diabetes mellitus; (c) 1 case refused to give a blood sample; and (d) 19 additional cases were not enrolled because sufficient study power was reached with the first thirty consecutives cases.

The characteristics of the individuals selected to enter the study protocol by group (cases / controls), age, sex, weight, height, BMI, blood total protein, albumin and globulin are summarized in Table 1. The mean and standard deviation of age for all individuals was 35.8 and 2.66. Thirty nine (66%; 39/59) were male. There were no significant differences between study groups for the following parameters analyzed: (a) age; (b) sex; (c) length; and (d) blood total protein. Weight (54.7 ± 8.3 *versus* 71.2 ± 14 kg; $p < 0.001$) and BMI (21 ± 2.8 *vs*. 26.2 ± 4.9; $p < 0.001$) were significantly lower among cases compared to controls (Table 1). While blood albumin concentration (4.1 ± 0.63 *vs*. 4.8 ± 0.28; $p < 0.001$) was significantly reduced, globulin concentration (3.5 ± 0.69 *vs*. 2.8 ± 0.33; $p < 0.001$) was increased in the tuberculosis group compared to healthy volunteers. The blood biochemistry substances measured, including glucose, liver and renal functional tests, total protein and fractions were within normal range for both cases and control groups.

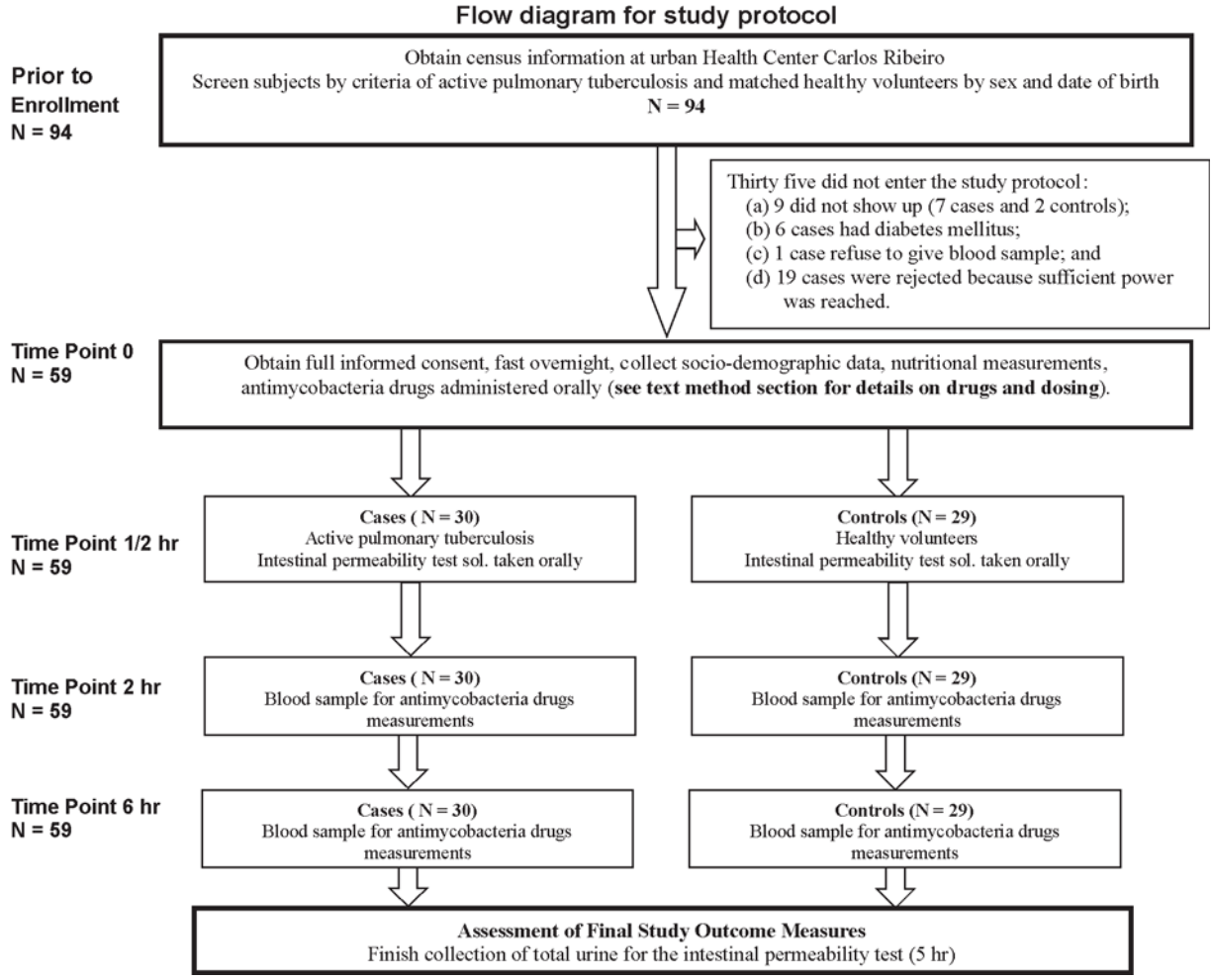
Fifty one percent (30/59) of all participants had alcohol dependence by the CAGE questionnaire evaluation criteria. The distribution of alcohol dependence in cases and controls were 60% (18/30) and 42% (12/29) and there was no significant difference between these groups ($p > 0.05$; Chi-square test). The results on intestinal permeability parameters are summarized in Table 2. Lactulose and mannitol are markers for the paracellular and transcellular transports, respectively, in the intestinal epithelium. The lactulose:mannitol ratio is also a parameter to measure intestinal permeability changes on intestinal barrier function [15,16]. The paracellular transport of lactulose was significantly reduced in cases (median: 0.2043% / range: 0.0-1.3015; $p = 0.0194$) compared to controls (0.4301% /0.0-2.0643). The mannitol transcellular transport (18.94% / 1.96-71.55 *vs*. 24.40% / 1.39-63.05; $p = 0.0698$) and the lactulose:mannitol ratio (0.0102 / 0.0-0.0760 *vs*. 0.0153 / 0.0-0.1360; $p=0.0698$) were borderline significantly reduced in cases compared to controls.

The percentage of total individuals with low serum maximum concentration for one or two antimycobacterial drugs was 27% (16/59) and 73% (43/59), respectively**.** The cases low serum concentrations were not significant difference from controls for isoniazid (cases: 83%; 25/30 *vs*. controls: 100%; 29/29; $p > 0.05$), but it reaches significant difference for rifampin (cases: 100%; 30/30 *vs*. controls: 62%; 18/29; $p < 0.001$).

Table 3 summarizes the data for delayed absorption of antimycobacterial drugs. Delayed absorption was defined as having the apparent maximum serum concentration at the six hour sample collection. Overall delayed absorption was observed in 48%, 21%, and 26% for rifampin, isoniazid and pyrazinamide, respectively. Although delayed absorption of isoniazid was more common with cases compared to controls, these differences were not statistically significant (Table 3).

Data on serum concentrations of antimycobacterial drugs for all participants, patients with active pulmonary tuberculosis, and healthy volunteers are summarized in Table 4 and Figure 2. For rifampin and isoniazid, the mean serum concentrations at 2 hours and at 6 hours after drug administration, as well as the maximum observed concentration were below the normal 2 h target ranges for all individuals combined, for cases, and for controls. For study subjects with maximum concentrations at 2 h, it was expected that the 6 h samples would be below the target ranges, especially for rifampin and isoniazid, because of their relatively short elimination half lives. However, given the high rate of delayed

Figure 1. Flow diagram for all individuals, patients with active pulmonary tuberculosis (cases) and healthy volunteers matched by date of birth and sex, Fortaleza, Ce, Brazil, from July, 2004 to December, 2005.



absorption (6 h > 2 h concentration) among study subjects, we also evaluated the 6 h samples against the target ranges. The mean serum concentrations at 2 hours post antimycobacterial drug administration, and the maximum observed concentration, were within the normal range for pyrazinamide. At six hours, the mean serum concentration for pyrazinamide was below the normal 2 h target value for all individuals and controls, but not for cases.

When analyses were done to compare the cases *versus* controls, we observed that the mean serum rifampin concentration was significantly lower in cases compared to controls for 2 hour and 6 hour samples. The maximum concentration of rifampin was also significantly lower in cases than controls. For rifampin, although the mean value for controls was considered low, the mean value for cases was considered very low. Isoniazid and pyrazinamide had maximum concentrations lower in controls than in the cases. The same was observed for pyrazinamide at 2 hours samples collected. One patient in the case group had history of diarrheal diseases in the last two weeks. For this case, the maximum serum concentration for rifampin (1.33 µg/mL) and isoniazid (2.23 µg/mL) was below the usual normal range. Pyrazinamide (29.31 µg/mL) was within the normal range maximum serum concentration.

Discussion

The five first-line drugs for tuberculosis treatment are still highly effective, with low rates of drug failure, and they have an acceptable degree of toxicity [5,30]. Short-course combined therapy for tuberculosis, as recommended by the World Health Organization, is based on a four drug regimen for the first two months and two drugs, rifampin and isoniazid, for the last four months [1]. This combined treatment relies on direct observation of patient compliance to ensure effective treatment and a high cure rate. The present study is consistent with previous literature, as we followed patients for more than

Figure 2. Scartter plot of the maximum serum concentrations of antimycobacteria drugs, the maximum serum concentrations within the normal range, and minimum inhibitory concentration by total individuals, patients with active pulmonary tuberculosis and healthy volunteers matched by date of birth and sex, Fortaleza, Ce, Brazil, from July, 2004 to December, 2005. Maximum serum concentration was defined as the highest serum concentration independent of serum sample time collected. Painel (**A**) shows the scartter plot of the rifampin (600 mg, v.o.) maximum serum concentration; (**B**) isoniazid (400 mg, v.o.) maximum serum concentration; and (**C**) pyrazinamide (2 g, v.o.). The plot shows also the mean ± standard deviation. The Student's unpaired *t* test was used to compare the mean between groups.

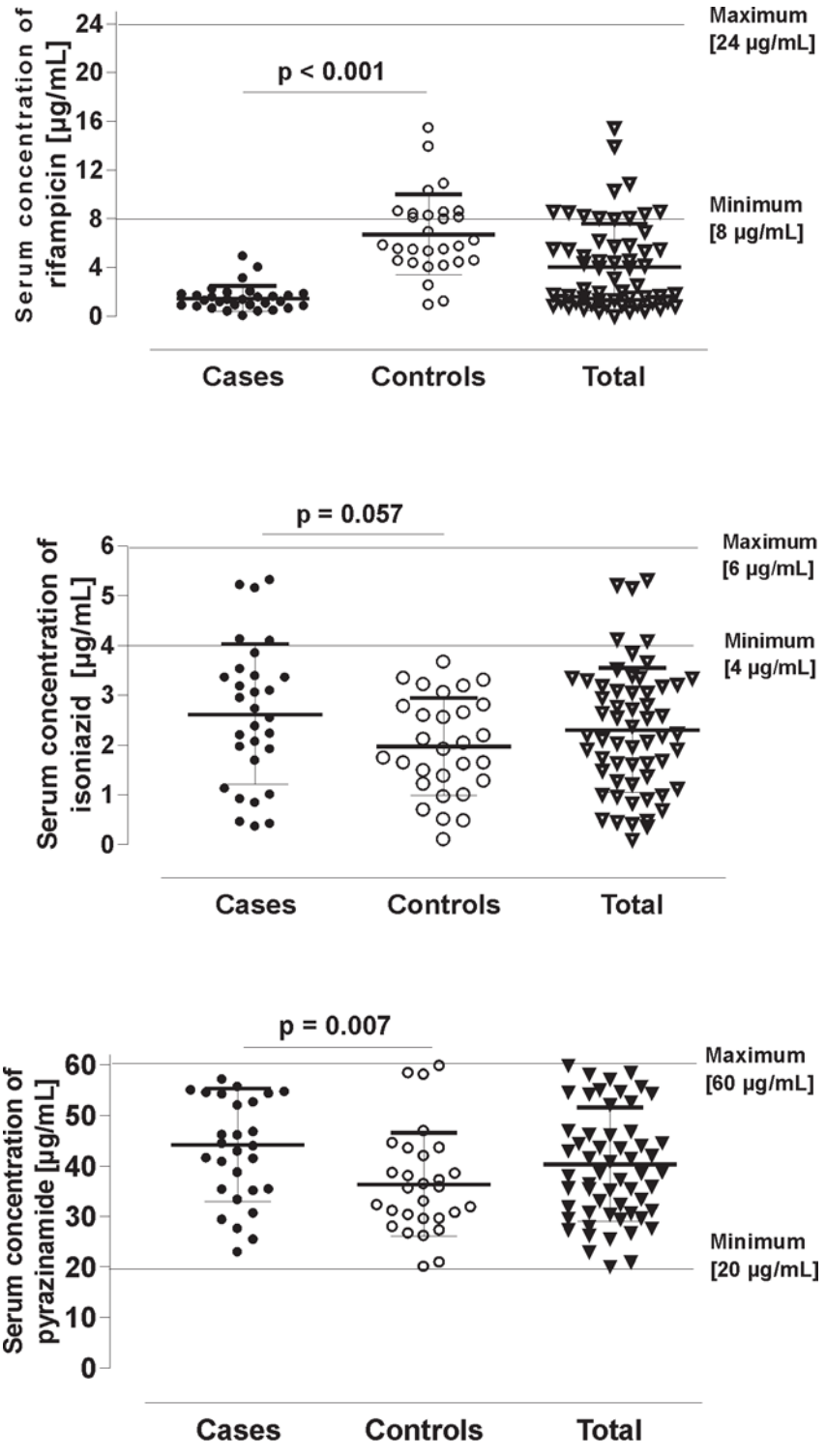


Table 1. The characteristics at baseline of the individuals selected to enter the study protocol by group (cases / controls), age, sex, weight, height, body mass index, blood total protein, albumin and globulin, Fortaleza, CE, Brazil, from July, 2004 to December, 2005.

Parameters	Total N = 59	Cases[1] N = 30	Controls N = 29	p[2] values
Age (Mean ± sd[2])	35.8 ± 13.0	34.5 ± 14.6	34.0 ± 11.0	0.294
Sex				
Female N (%)	20 (33.9)	10 (33.3)	10 (34.5)	0.926
Male N (%)	39 (66.1)	20 (66.7)	19 (65.5)	0.926
Weight (kilogram)	62.8 ± 13.8	54.7 ± 8.3	71.2 ± 14.0	<0.001
Height (meter)	1.63 ± 0.09	1.62 ± 0.07	1.65 ± 0.1	0.438
BMI[4] (kg / m²)	65.7 ± 12.1	60.3 ± 8.0	71.2 ± 14.0	0.038
Total proteins (mg / dL)	7.55 ± 0.8	7.55 ± 0.93	7.56 ± 0.46	0.538
Albumin (mg / dL)	4.42 ± 0.69	4.08 ± 0.63	4,78 ± 0.28	<0.001
Globulin (mg / dL)	3.12 ± 0.68	3.47 ± 0.69	2.76 ± 0.33	<0.001

[1] Cases = active pulmonary tuberculosis; controls = healthy volunteers matched by sex and date of birth. [2]*P* Value of significance by Student's unpaired t test or Chi-square test. [3] sd = Standard deviation. All data are shown as mean ± sd unless different indicated. [4] BMI = body mass index = weight / height square.

Table 2. Intestinal permeability test as measured by the percentage of urinary excretion of lactulose, mannitol and lactulose:mannitol ratio in the total individuals, active pulmonary tuberculosis and healthy volunteers matched by date birth and sex, Fortaleza, Ce, Brazil, from July, 2004 to December, 2005.

Parameters	Total (N = 59) Median (Range)	Cases[1] (N = 30) Median (Range)	Controls (N = 29) Median (Range)	p[2] values
% Lactulose[3]	0.3066 (0.0-2.0643)	0.2043 (0.0-1.3015)	0.4301 (0.0-2.0643)	0.0194
% Mannitol[4]	21.65 (1.39-71.54)	18.94 (1.96-71.55)	24.40 (1.39-63.05)	0.0698
Lactulose:Mannitol ratio	0.0140 (0-0.1360)	0.0102 (0.0-0.0760)	0.0153 (0.0-0.1360)	0.0698

[1] Cases mean active pulmonary tuberculosis; controls mean healthy volunteers matched by date of birth and sex. [2] p Values of significance by Mann-Whitney test. [3] Percentage of urinary excretion of lactulose. [4] Percentage of urinary excretion of mannitol.

Table 3. Distribution of delayed absorption of antimycobacteria drugs by total individuals, patients with active pulmonary tuberculosis and healthy volunteers matched by date of birth and sex, Fortaleza,Ce, Brazil, from July, 2004 to December, 2005.

Drugs[1]	Total (58) N (%)	Cases (29)[2] N (%)	Controls (29) N (%)	p values[3]
Rifampin	28 (48)	12 (41)	16 (55)	0.431
Isoniazid	12 (21)	9 (31)	3 (10)	0.103
Pyrazinamide	15 (26)	7 (24)	8 (28)	1.000

[1] Delayed drugs absorption were defined as maximum serum concentration found at 6 hour sample collected. [2] One case did not have enough samples for the six hours drugs measurements. [3] p Values of significance by Fisher exact test.

six months (data not shown) and observed a ninety percent cure rate (27/30; two gave up the treatment and one had failed the treatment) among our patients with active pulmonary tuberculosis using this standard combined therapy. Relapse post-therapy was beyond the scope of this study.

Reports have warned of the relative high frequency of mutants resistant to rifampin, isoniazid and the risk of multidrug resistant tuberculosis (MDR-TB) [6,7,31,32]. Although the prevalence of MDR-TB is still low worldwide, the ascendant rate of increase is very dramatic [6,7]. In Fortaleza, capital of the state of Ceará in the Northeast of Brazil, the percentage of MDR-TB has increased from 0.82% (1994) to 1.48% (1999) [7]. The issue of low serum concentrations of antimycobacterial drugs and the relationship to delayed or incomplete response to treatment, or emergence of drug resistance is still not totally understood. Only a few studies have been conducted on the pharmacokinetics of first choice drugs for tuberculosis treatment [10-17]. In this report we observed peak serum concentrations of rifampin and isoniazid that were below normal range for these drugs. This is consistent with other

Table 4. Serum sample concentrations of antimycobacteria drugs in the total individuals, active pulmonary tuberculosis and healthy volunteers matched by sex and date of birth, Fortaleza, Ce, Brazil, from July 2004 to Dedember, 2005.

Drugs	Total (N = 59)	Cases[1] (N = 30)	Controls (N = 29)	p values[2]
Rifampin at 2 hours	3.13 ± 3.49	1.15 ± 0.78	5.07 ± 3.9	0.0001
Rifampin at 6 hours	3.13 ± 2.57	1.18 ± 0.51	5.07 ± 1.55	<0.001
Rifampin maximum[3]	3.97 ± 3.58	1.46 ± 0.72	6.69 ± 3.07	<0.001
Isoniazid at 2 hours	2.36 ± 1.24	2.30 ± 1.60	1.86 ± 0.75	0.194
Isoniazid at 6 hours	0.95 ± 0.74	1.40 ± 0.62	0.96 ± 0.86	0.109
Isoniazid maximum	2.46 ± 1.19	2.62 ± 1.53	1.98 ± 0.76	0.057
Pyrazinamide 2 hours	40.17 ± 12.42	42.38 ± 11.02	35.11 ± 13.16	0.015
Pyrazinamide at 6 hours	28.72 ± 5.78	31.80 ± 4.99	27.99 ± 6.46	0.129
Pyrazinamide maximum	40.94 ± 11.44	44.10 ± 10.40	36.32 ± 12.02	0.007

[1] Cases = active pulmonary tuberculosis; controls = healthy volunteers matched by date of birth and sex. [2] p Value of significance by Student's unpaired t test. All data (mg/mL) are presented as mean ± standard deviation. [3] Maximum serum concentrations were defined as the highest measurement independent of the sample time collected.

studies using different populations with pulmonary tuberculosis and HIV/AIDS [10-17]. The appropriate absorption of pyrazinamide observed in this study is also consistent with other reports [10-17].

New in the literature, and against our primary hypothesis, are the findings of low serum concentrations for these drugs in healthy volunteer controls. Thus, low serum peak concentrations of antimycobacterial drugs were not dependent on tuberculosis itself or other risk factors evaluated in this study, including malnutrition, alcohol abuse, total protein and albumin, and intestinal barrier function.

Two previous studies have suggested that small absorptive intestinal surface area could be the primary reason for the low drug absorption in these populations [10,13]. However, the absence of healthy controls in these reports did not allow completely for this assumption. Malnutrition, low blood albumin concentrations and altered intestinal barrier function were also confirmed in the present study for patients with active pulmonary tuberculosis, but these could not explain the low drugs serum concentrations seen also in healthy control individuals.

The drug content *in vitro* was evaluated by a laboratory independent of the manufacturer and found to be as labeled, thus the drug amount in the medication was not considered the primary cause for the observed low serum drugs concentrations. Since all individuals were requested to fast prior to medication administration, the influence of food on drug absorption was not likely to be the cause. Blood samples were collected and, within less than an hour preserved at appropriate temperature, as recommended for the quality control measurements of these drugs [26-28]. The blood biochemistry substances measured allowed us to eliminate diabetes mellitus, and liver or kidney disease as possible confounding factors.

The proportion of cases with delayed absorption of drugs was not significantly different from controls, and a relatively high proportion (Table 4) of both cases and controls had delayed peak drug concentrations. There were no samples collected between 2 and 6 hours to evaluate an intermediate time to maximum concentrations. The first pass effect of drug biotransformation at the intestinal wall could not be ruled out and is a limitation of this study.

Although cases had weight and body mass index measurements that were significantly lower than controls, the percentage of those identified as underweight was only 16% (5/30). In addition, the area of absorption, as measured by the mannitol transcellular marker, had only a marginally significant lower absorption area than controls which does not explain the low drugs absorptions for both cases and controls. Alcohol abuse is another potential cause of reduced drug absorption and it could have contributed in cases and controls to reduce intestinal drug absorption and produce low serum concentrations [33].

Paracellular intestinal transport, as measured by lactulose marker, was significantly lower in cases than in controls. However, this pathway should not significantly influence drug absorption, since it represents less than five percent of the total area of absorption in the small intestine [34]. In addition, it was not associated with low peak serum concentration in total individuals, cases, or controls.

In conclusion, these results showed a high proportion of individuals (cases and controls) with low peak serum concentrations for rifampin and isoniazid. Pyrazinamide was well absorbed and had normal range serum peak concentration in cases and controls. The intestinal transcellular and paracellular transport and the lactulose:mannitol ratio were all significantly or borderline reduced in active pulmonary tuberculosis, and these markers were not associated with low serum peak rifampin and isoniazid concentrations. These data warrant further studies investigating the efficacy of current guidelines for normal peak serum concentrations and dosing of rifampin and isoniazid for combined therapy of tuberculosis in different populations around the world.

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