Intestinal Permeability and Malabsorption of Rifampin and Isoniazid in Active Pulmonary Tuberculosis

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Low antimycobacterial drug concentrations have been observed in tuberculosis (TB) patients under treatment. The lactulose/mannitol urinary excretion test (L/M), normally used to measure intestinal permeability, may be useful to assess drug absorption. The objective of this research was to study intestinal absorptive function and bioavailability of rifampin and isoniazid in TB patients. A cross sectional study was done with 41 patients and 28 healthy controls, using the L/M test. The bioavailabilities of rifampin (R) and isoniazid (H) were evaluated in 18 patients receiving full doses. Urinary excretion of mannitol and lactulose, measured by HPLC, was significantly lower in TB patients. The serum concentrations of the drugs were below the expected range for R (8-24 mcg/mL) or H (3-6 mcg/mL) in 16/18 patients. Analyzing the drugs individually, 12/18 patients had low serum concentrations of R, 13/18 for H and 8/18 for both drugs. We suggest that there is a decrease in the functional absorptive area of the intestine in TB patients, which would explain the reduced serum concentrations of antituberculosis drugs. There is a need for new approaches to improve drug bioavailability in TB patients.

Key Words: Pulmonary tuberculosis, intestinal permeability, rifampin and isoniazid intestinal malabsorption; *M. tuberculosis* resistance.

Concentrations of antimycobacterial drugs that are below those normally expectedduring tuberculosis (TB) therapy may predispose for the appearance of resistant strains of *Mycobacterium tuberculosis* [1,2]. Among the reasons often given for low antimycobacterial drug concentrations are inadequate dosing and irregular drug intake. However, other factors may compromise the bioavailability of anti-TB drugs [3-6], including malabsorption due to intestinal function impairment.

Under normal conditions, rifampin (R) and isoniazid (H) are usually well absorbed when administered orally. The usual doses of 600 mg (R) and 400 mg (H), taken fasting, will reach the maximum serum concentrations (Cmax) of 8 to 24 mcg/mL and 3 to 6 mcg/mL, respectively, within two hours [5,7,8]. However, intestinal absorption of these drugs can take longer, up to five hours, or be incomplete when they are consumed with food or antiacids [9-11].

Nevertheless, the determining factor for drug absorption is the absorptive capacity of the intestinal mucosa, which may be altered in several clinical situations [12-14]. HIV-infected TB patients may have low serum concentrations of antiretroviral drugs [15] and also may have alterations in the pharmacokinetic profile of anti-TB drugs [16-19]. Among the drugs commonly used for TB treatment, rifampin (R) and ethambutol (E) appear to be the ones with the greatest reduction in intestinal absorption by the HIV^+/TB patient. In HIV^+/TB patients with diarrhea, isoniazid (H) absorption is notably reduced, while the absorption of pyrazinamide (Z) does not appear to be as severely affected [20].

Several authors [1,8,21-24] suggest that malabsorption of anti-tuberculosis drugs occurs in 2% to 5% of patients, despite a lack of evidence. Intestinal malabsorption should be considered as a possible reason for failure or relapse in patients who adhere to appropriate treatment and drug intake.

Pharmacokinetic studies of drug absorption are scarce [25], especially in patients with TB. Using the D-xylose absorption test as a method to evaluate intestinal permeability, Choudhri et al. [20] showed that intestinal absorption was directly correlated with rifampin and isoniazid bioavailability in TB patients. Gurumurthy et al. [26] found that urinary excretion of D-xylose after oral administration correlated with reductions in urinary excretion of rifampin and isoniazid as well as their respective metabolites, desacetyl rifampin and acetyl isoniazid in patients with HIV infection plus diarrhea and in those with HIV infection plus TB.

The differential urinary excretion of ingested lactulose and mannitol (L/M test), as functional markers of barrier disruption and the overall intestinal villous surface, respectively, has

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been widely used to measure paracellular intestinal permeability and absorptive surface [27-29]. Some studies using the L/M test showed impaired intestinal permeability and absorption in HIV-infected patients with chronic diarrhea treated in the São José Hospital, Fortaleza, CE, Brazil [30].

We looked for possible correlations of intestinal permeability, based on the L/M test, of serum concentrations of rifampin and isoniazid in HIV-seronegative patients with pulmonary TB

Material and Methods

Study design and population

The study was carried out in compliance with the Helsinki Declaration (1965) and was approved by the Research Ethics Committee of the Federal University of Ceará (UFC). This study was conducted from July 2001 to December 2002 at the Maracanaú Hospital, the state reference hospital for TB in Ceará, Brazil. The participants consisted of 41 HIV-seronegative patients with active pulmonary TB. They were required to meet the following criteria: positive direct microscopy for acid-fast bacteria in at least one sputum sample and antituberculosis drug intake for at least two months; age over 15 years; no significant hepatic or renal dysfunction (i.e., liver enzymes, blood urea, and serum creatinine levels within normal limits); not being diabetic, and no gastrointestinal disease or any medical illness that might interfere with drug pharmacokinetics. Pregnant and HIV-seropositive patients were excluded. All participants gave informed written consent.

In addition, a control group of 28 healthy individuals living in the same area and with matching ages were recruited for intestinal permeability tests. Eighteen patients taking a full dose of R+H+Z (scheme I) were selected for 600 mg rifampin and 400 mg isoniazid dose intake (NUPLAN, Natal, Brazil) under fasting conditions and direct observation in order to assess rifampin and isoniazid bioavailability.

The WHO reference values [31] of the body mass indices (BMI) were used to make nutritional status assessment of all participants.

Determination of intestinal permeability

To carry out the L/M test, the patients were instructed to empty their bladders before taking a 20 mL oral dose of a 250 mg/mL lactulose and 50 mg/mL mannitol solution (L/M test) (LABIOTEC/IBIMED, Fortaleza, Brazil). Urine excreted up to 5h after L/M test intake was obtained; the volume was measured, and 5 mL aliquots were stored at -80°C. The sugars in the urine were detected and quantified by high-pressure liquid chromatography with anionic exchange coupled with pulsed amperometric detection (HPLC-PAD) [28]. Patients were free to have breakfast one hour after drug and test-solution intake. The healthy volunteers followed the same clinical protocol for L/M permeability testing used for comparison with study patients.

Determination of serum concentrations of rifampin and isoniazid

An 8 mL blood sample was obtained from the 18 patients to determine the maximum serum drug concentrations (C Max T_{2h}). Samples were collected in heparinized tubes two hours after the R and H medications were taken. Drug concentrations were assayed using an HPLC at the Infectious Disease Pharmacokinetics Laboratory (IDPL) at National Jewish Research Medical Center (NJRMC), Denver CO. Based on pharmacokinetic studies, serum concentrations at 2h post dose of rifampin at 8-24 mcg/mL and isoniazid at 3-6 mcg/mL were considered "normal" [2]. The quality of expected measurable anti-tuberculosis drugs (R and H) used in this study was assayed at the IDPL by thin-layer chromatography.

Sample size calculation

The lactulose/mannitol ratio was selected as the primary outcome variable. Based on data from previous studies in Fortaleza [30], we expected that the patient group would have a 30% reduction in the lactulose/mannitol ratio, compared to healthy controls. Using a power of 80% and a two-sided significance level of 5%, a sample size of 23 for each group was considered adequate to detect a difference in the L/M ratio between groups. We assumed a possible loss of 10% and thus estimated at least 26 subjects in each group.

Statistical analysis

The data were double-taped and validated by cross-checking using Excel software version 7.0 (Microsoft Corporation, Redmond, WA). Analysis of data was performed using Statistical Package for Social Sciences software version 11.5 (SPSS, Chicago, IL). The Mann-Whitney test was used to compare the intestinal permeability test results between the groups (TB *versus* control). Differences were considered significant if $P < 0.05$.

Results

Thirty patients were male and 11 female, with an average age of 42.8 years (range: 15 to 76 years). Thirty-two patients were in the tisiology ward of Maracanaú Hospital; the other nine were outpatients in treatment at the tisiology unit during the same period. Controls included 15 men and 13 women, with average age 34.5 (range: 16 to 79 years).

The mean weight ± sd for TB patients was 47.5 ± 10.5 kg; the mean height was 1.60 ± 0.08 meters and the mean BMI = 18.2 ± 3.2 kg/m^2. Twenty-four of the 41 patients were considered malnourished (BMI < 18.5 kg/m^2); nine of these were considered severely malnourished (BMI <16 kg/m^2); and 17 were considered well nourished. The group of pulmonary TB patients had significantly lower body weights, heights and BMI than the healthy controls (Table 1).

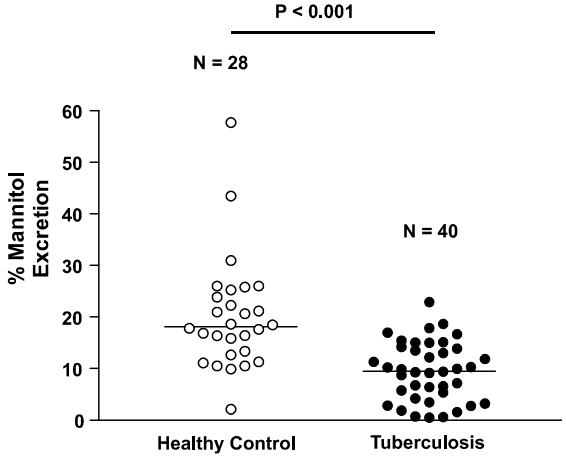
The lactulose/mannitol test was well tolerated; one patient failed to provide urine over the 5h collection period and

Table 1. Weight, height and body mass index of individuals submitted to an evaluation of intestinal permeability with the mannitol/lactulose test

Variable	Healthy controls (n= 28)	Patients with active pulmonary tuberculosis (n = 41)	P values[§]
Weight (kg; mean ± SD)	67.3 ± 13.4	47.5 ± 10.5	< 0.001
Height (m; mean ± SD)	1.66 ± 0.10	1.60 ± 0.08	< 0.006
BMI[*] (kg/m^2; mean ± SD)	24.1 ± 3.9	18.2 ± 3.2	< 0.001

[§]Comparison of patients with active pulmonary tuberculosis *versus* healthy controls by independent Student *t* test. [*] BMI = Body Mass Index.

Figure 1. Scatter data and median of the percentage of urinary excretion of mannitol in healthy controls and in patients with active pulmonary tuberculosis. The Mann-Whitney test was used to compare controls *versus* patients with tuberculosis (P < 0.001). The data suggest a significant decrease in absorption area in patients with pulmonary tuberculosis.



lactulose was not detected in 18 urine specimens of the patients. The urinary excretion of mannitol, lactulose and the lactulose/mannitol ratio are shown in Table 2.

The percentage of urinary excretion of mannitol was significantly lower (P<0.001) in patients (median /range = 9.66/ 0.52-22.91) than in the controls (median /range = 18.14/2.15-57.73) (Figure 1). Excretion of lactulose was also significantly lower in patients (P < 0.05) (median /range = 0.04 /0.0-8.56) than in controls (median /range = 0.39 /0.6-2.09), and the L/M ratio was slightly, but not significantly, lower in patients when compared with controls (Table 2).

The rifampin and isoniazid used in the study were considered pharmaceutically equivalent to rifampin and isoniazid USP. The median/range rifampin concentrations were 6.47/0-31.95 mcg/mL), and the median/range isoniazid concentrations were 2.17/0.8-3.77 mcg/mL). Considering the expected "normal" concentrations of R (8-24 mcg/mL) and H (3-6 mcg/mL), 16/18 patients had low concentrations of one or both drugs, 12/18 patients had low serum concentrations of rifampin (CRM2h) and 13/18 had low isoniazid (CINH2h) levels (Figures 2A and B). In eight of the patients, both drug concentrations were low.

Discussion

Alterations in intestinal permeability, allowing the passage of macromolecules in cases of bronchial asthma, atopical eczema and sarcoidosis, among others, have been increasingly recognized [32,33]. In pulmonary tuberculosis, little is known about the role of intestinal permeability in drug absorption.

At present, there is no gold standard among the investigation methods for intestinal permeability, which hampers the comparison of published results; hence inclusion of control groups remains important for the interpretation of alterations in intestinal permeability in different situations.

Some factors that influence intestinal absorption can be excluded in our study, such alterations in the osmolarity of the test solution, since it was prepared strictly under the same conditions, and gastrointestinal diseases that alter peristaltic speed, since these diseases were included as exclusion criteria. The groups were matched by age, although gender and age do not normally alter absorptive function.

In our study, using the lactulose/mannitol test, intestinal permeability in patients with pulmonary tuberculosis was clearly abnormal. Significant reductions in urinary excretion of mannitol and lactulose were observed in TB patients, when compared to controls. The L/M ratio, although decreased in the patients, did not vary significantly between groups.

Among the patients, a high percentage of L/M tests was found in which lactulose was undetectable, but not in the controls, which we also found in a previous study [30]. There is no clear explanation for these results. In order to look for differences among patients regarding measurable lactulose, the two groups of patients were compared: group A (lactulose = 0) and B (lactulose > 0) for anthropometric data, inflammatory markers and the presence of intestinal parasites. None of the parameters showed differed significantly between the groups (P>0.05), so we considered the result of undectectable levels of lactulose.

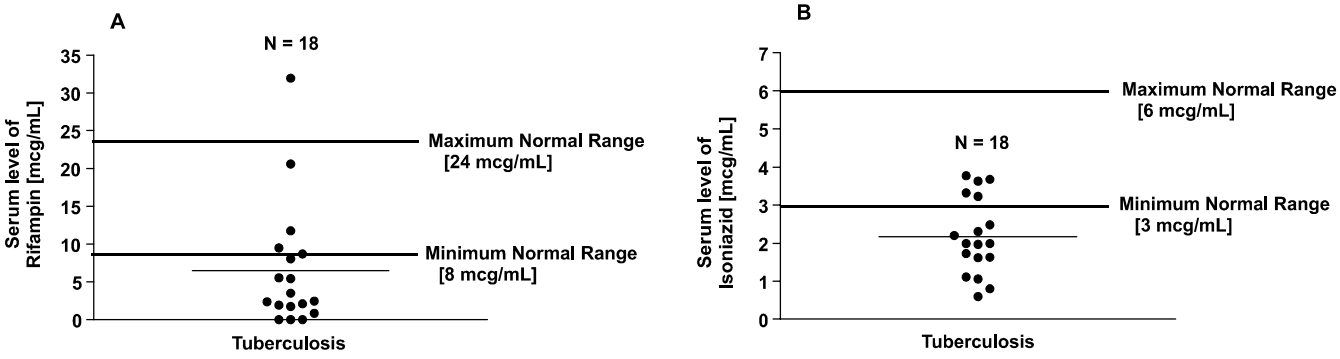
It is known that the intestinal absorption routes of these two sugars are different; lactulose has paracellular absorption through the more permeable intercellular tight junctions, and mannitol has transcellular absorption, possibly through the enterocytes [27]. If the urinary excretion rate of mannitol is low, the absorption of small molecules may be compromised and a reduction in the area of absorptive mucosa can be inferred. If the rate of lactulose

Table 2. Descriptive amounts of the urinary excretion of mannitol, lactulose and the lactulose / mannitol ratio in patients with active pulmonary tuberculosis and in healthy controls

Intestinal permeability parameters	Controls Median (Range)	Patients with tuberculosis Median (Range)	P value
% Mannitol*	18.14 (2.15-57.73)	9.66 (0.52-22.91)	<0.001
% Lactulose**	0.39 (0.6-2.09)	0.04 (0.0-8.56)	<0.05
Lactulose/Mannitol ratio***	0.02 (0.1-0.07)	0.01 (0.0-0.51)	NS

Mann-Whitney Test; NS means not significant (P > 0.05). *Percentage of urinary excretion of mannitol (patients n = 40; controls n = 28). **Percentage of lactulose urinary excretion (patients n = 40; controls n = 28). ***Ratio of the urinary excretion of lactulose:mannitol (patients n = 40; controls n = 28).

Figure 2. Distribution of the serum levels after two hours of ingestion of 600 mg rifampin (CRM2h) (A) and 400 mg isoniazid (CINH2h) (B) in 18 patients with active pulmonary tuberculosis in Maracanaú Hospital July 2001 – December 2002.



excretion is high, this indicates an increase in intestinal permeability to large molecules, which may be due to a disruption of the selective intestinal barrier regulated by the zonula occludens (tight junctions) between enterocytes. In contrast, if lactulose excretion is low without cell damage, this condition could reflect a decrease in conductance throughout the tight junctions. This may have occurred in the TB patients in our study. Recently, an increasing number of cytokines have been shown to influence tight junction function, both *in vitro* and *in vivo*. Cytokine-induced effects on tight junction barrier function have also been correlated with effects on intrinsic tight junction proteins and on the associated actin cytoskeleton [34]. Reduced mannitol excretions, along with reduced lactulose excretion, could explain why the lactulose/mannitol ratio was not significantly different between TB patients and healthy controls in our study.

The findings suggest that the intestinal absorptive area was reduced in the TB patients. These results are consistent with prior accounts of intestinal malabsorption in tuberculosis patients [1,26,35].

It could be questioned whether a single sample collected two hours after the ingestion of the drugs can provide a measure of the Cmax. In fact, studies have shown that Tmax for both rifampin and isoniazid occurs around two hours post dose [2,21], although Cmax may peak before or after this period, particularly in patients with altered patterns of intestinal absorption. So, the two-hour dosing measure could detect altered drug absorption but would be inadequate to distinguish between delayed absorption and malabsorption [11].

Another issue to be discussed is the influence of residual concentrations of drugs in TB patients under treatment. Peloquin's studies [2] showed that even with daily doses, seven half-lives occur between doses, so that more that 99% of the drug is eliminated during 24 hours, except in the case of patients with hepatic disease.

Mehta et al. [23] state that patients with pulmonary tuberculosis are frequently malnourished and have hypoalbuminemia. The decreased plasma proteins could reduce plasma protein binding of drugs, consequently making more drugs available for clearance. Although isoniazid is not highly protein bound, rifampin is approximately 85% protein bound, and might be somewhat affected by this alteration. In our study observed 43% of the patients had < 3.5g/dL serum albumin. However, this is insufficient to explain the reductions in drug concentrations.

In summary, we found a significant decrease in the urinary excretion of mannitol and lactulose in TB patients, when compared to controls, and we found reduced serum concentrations of rifampin and isoniazid in these patients. A significant correlation between the serum concentrations of drugs with mannitol, lactulose or lactulose/mannitol ratios was not found, probably due to the small number of patients

in each category.In conclusion, the findings of impaired absorptive function and reduced concentrations of antituberculosis drugs point to a need for further study of the underlying mechanisms, their impact on resistance, and potential therapeutic approaches to improve intestinal function and drug absorption in patients with tuberculosis, especially in areas where drug resistant tuberculosis is an emerging problem.

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