Malabsorption of Rifampin and Isoniazid in HIV-Infected Patients With and Without Tuberculosis

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The absorption of rifampin, isoniazid, and D-xylose in patients with human immunodeficiency virus (HIV) infection and diarrhea, in patients with HIV infection and tuberculosis (TB), in patients with pulmonary TB alone, and in healthy subjects was studied. Percentage of dose of the drugs, their metabolites, and D-xylose excreted in urine were calculated. A significant reduction in the absorption of drugs and D-xylose in both the HIV infection/diarrhea and HIV infection/TB groups was observed ($P < .05$), and the correlation between them was significant. Our results indicate that patients with HIV infection and diarrhea and those with HIV infection and TB have malabsorption of rifampin and isoniazid.

Although most HIV-infected patients with tuberculosis (TB) respond well to rifampin-based antimycobacterial drug regimens [1], recent reports suggest that malabsorption of antimycobacterial drugs occurs in selected HIV-infected patients, particularly those with advanced HIV infection [2–6]. In theory, the resulting lower drug exposure may contribute to acquired drug resistance and reduced effectiveness of anti-TB treatment [7]. The degree of drug malabsorption appears to be more pronounced in North American populations [6, 8] than in African populations [9, 10]. Many factors might contribute to this difference across studies and across populations, including variations in the type and severity of concurrent gastrointestinal infection. Although it is not known which specific factor or combination of factors produce drug malabsorption, it remains reasonable to consider this in patients with concurrent TB and HIV infection.

Information on absorption of anti-TB drugs in HIV-infected subjects in India is not available. Therefore, an investigation to study the absorption of rifampin and isoniazid (based on the urinary excretion of the drugs and their metabolites) in HIV-infected patients with and without TB was undertaken. The D-xylose absorption test was also performed to assess the absorptive capacity of the intestines.

Material and methods. The study was conducted at the Government Hospital of Thoracic Medicine (Tambaram, Chennai, India). All study participants were men and were admitted to the hospital for clinical problems. They were required to meet the following criteria: they were 18–50 years of age; no significant hepatic or renal dysfunction (i.e., liver enzymes, blood urea, and serum creatinine levels were within normal limits) was present; they were not diabetic; they did not have a coexisting medical illness that might interfere with drug pharmacokinetics, apart from HIV infection and diarrhea; and they were willing to provide informed written consent.

The participants consisted of 23 HIV-seronegative patients with pulmonary TB (group 1); 40 patients with advanced HIV infection and diarrhea (group 2); 26 patients with HIV infection and TB, 20 of whom had diarrhea (group 3); and 10 healthy volunteers (group 4). Participants in group 2 had a history of recurrent episodes of watery stools (i.e., 6–8 watery stools per day for ≥10 consecutive days in 1 month) at the start of the study. Examination of stool specimens for detection of opportunistic enteric pathogens was performed for all patients with diarrhea. Stool specimens were stained with a modified acid-fast bacillus stain but were not cultured for mycobacteria. Pulmonary TB was diagnosed on the basis of bacteriological investigations (sputum smear and culture for mycobacteria), which were supported by clinical and radiological features. Diagnosis of HIV infection was based on 3 positive results of tests (2 rapid tests [Tridot {Mitra and Co.} and Combaids {Span Diagnostics}], followed by an ELISA [Labsystems]).

All participants in groups 1 and 3 except 1 participant in group 3 were sputum-smear positive for acid-fast bacilli and were receiving standard anti-TB regimens. Mycobacterial cultures were performed only for 16 patients in group 3; *Mycobacterium tuberculosis* was isolated for 15 patients. One patient in group 3 had extrapulmonary TB with pleural effusion. None of the patients in group 1 complained of diarrhea or vomiting for ≥3 days before the start of the study. None of the HIV-seropositive patients in groups 2 and 3 were receiving antiret-

roviral treatment, but they were receiving medications for other opportunistic infections, in addition to multivitamins. None of these patients were receiving any stool binders, such as pectin or kaolin, or were receiving any medications known to interfere with the absorption of isoniazid or rifampicin. The healthy volunteers were staff and students of the Tuberculosis Research Centre (Chennai). The study was conducted after obtaining approval from the Ethics Committee of the health care center, and informed written consent was obtained from each participant.

Baseline demographic, clinical, and laboratory data were obtained from all eligible study patients. Rifampin and isoniazid were withheld for a period of 48–72 h before the start of the study. All participants received rifampin (450 mg) and isoniazid (300 mg) orally under supervision. Two hours later, a uniform oral dose of D-xylose (5 g) in water was administered. Urine excreted up to 8 h after drugs were administered was obtained; the volume was measured, and aliquots were stored at −20°C. Ascorbic acid was added to urine aliquots to prevent oxidation of rifampin. The D-xylose absorption test was performed for 10 healthy volunteers (mean age, 38.0 years; mean body weight, 65.4 kg).

The concentration of rifampin and its primary metabolite, desacetyl rifampin (DRMP), were measured by a high-performance liquid chromatography method developed in our laboratory [11]. The concentration of isoniazid and its metabolite, acetyl isoniazid (AcINH) [12], and that of D-xylose were measured by spectrophotometric methods [13]. The values were expressed as percentage of dose of rifampin (rifampin and DRMP), isoniazid (isoniazid and AcINH), and D-xylose excreted in urine. All estimations were undertaken after coding the samples.

Analysis of data was performed using the SPSS software package, version 10.5 (SPSS). The significance of differences in mean percentage of dose between the 2 study groups was evaluated using an unpaired Student's t test, and analysis of differences between >2 groups was performed using analysis of variance. Significance was defined at the 5% level. Pairwise correlation between excretion of D-xylose, rifampin, and isoniazid and CD4 lymphocyte counts was calculated using Pearson's correlation method.

Results. The baseline demographic characteristics of all of the patients are shown in table 1. CD4 lymphocyte counts were available for only 17 patients each in groups 2 and 3, and the mean values were 123 and 61 cells/mm³, respectively. The mean CD4 lymphocyte count was significantly lower in group 3 than in group 2. Examination of stool specimens revealed *Cryptosporidium parvum* in almost all of the cases in groups 2 and 3. Many patients had mixed infection, including infection with *Candida* species, *Ascariasis* species, *Hymenolepis nana,* and *Entamoeba histolytica.* None of the patients had tubercle bacilli detected on stool smear. The renal function of the patients in all 4 study groups was normal.

The mean percentage of dose of D-xylose excreted in urine for patients with TB was 30.2, which was not significantly different from that for the healthy volunteers (30.0). Therefore, for additional comparison with respect to drug levels, group 1 was considered to be the control group with which groups 2 and 3 were compared. The mean percentages of doses of D-xylose for groups 2 and 3 were 17.0 and 20.9, respectively, both of which were significantly lower than that for group 1 ($P <$ .001) (figure 1*A*).

A significant reduction in the mean percentage of dose of rifampin and DRMP excreted was observed in groups 2 and 3, compared with group 1 (7.4, 6.6, and 10.1, respectively; $P <$ .01). Excretion of rifampin and DRMP was reduced by 27% and 34% in groups 2 and 3, respectively (figure 1*B*).

The mean percentages of doses of isoniazid and AcINH excreted in groups 1, 2, and 3 were 49.6, 37.9, and 38.3, respectively. A significant decrease in the excretion of isoniazid and AcINH was observed in groups 2 and 3, compared with group 1 ($P <$ .05): the mean reduction in the excretion of the drug in groups 2 and 3 was 24% and 23%, respectively (figure 1*C*). No significant differences in the urinary excretion of D-xylose, rifampin, and isoniazid were observed between groups 2 and 3.

A significant positive correlation existed between percentage of dose of D-xylose and that of rifampin and isoniazid in urine (r = 0.55 and 0.49, respectively; $P <$ .001). However, the CD4 lymphocyte counts correlated significantly only with percentage of dose of rifampin (r = 0.38; $P <$ .01). Also, the correlation between percentage of dose excreted of rifampin and of isoniazid was significant (r = 0.34, $P <$ .01).

Discussion. Tuberculosis is the most common opportunistic infection in HIV-infected patients in India. Despite initial

Table 1. Characteristics of patients in the different study groups.

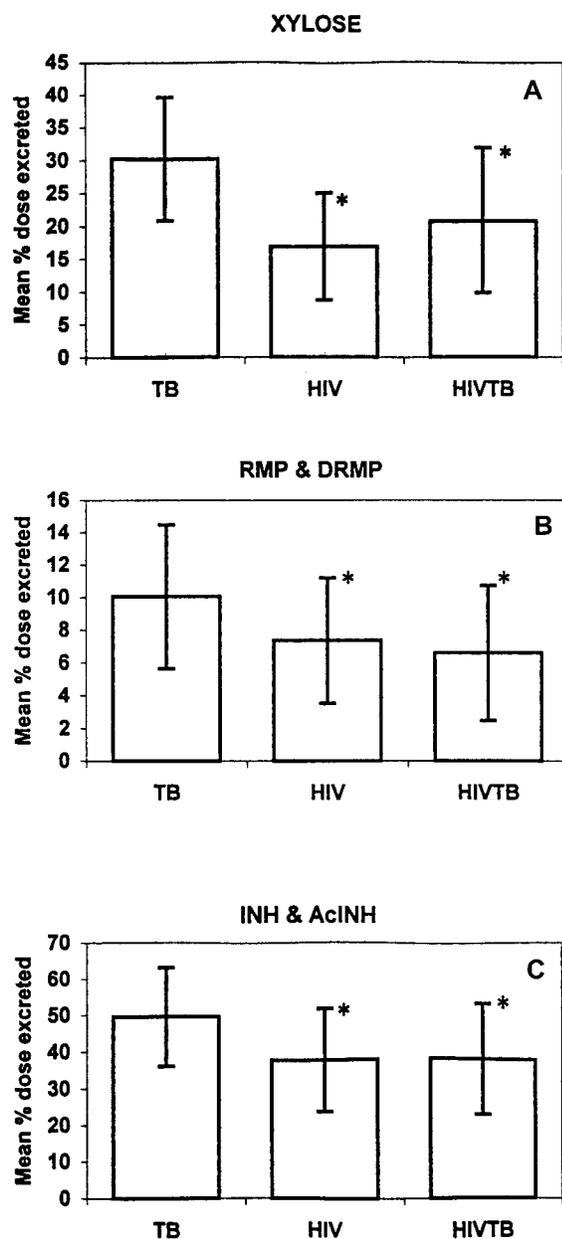| Group	No. of patients	Mean value ± SD	
		Age, years	Body weight, kg
Patients with TB	23	41.2 ± 6.8	40.1 ± 7.1
HIV-infected patients with diarrhea	40	33.7 ± 6.3	42.8 ± 6.0
HIV-infected patients with TB	26	36.7 ± 7.0	43.3 ± 6.6

NOTE. TB, tuberculosis.

Figure 1. The mean percentage of dose of D-xylose *(A)*, rifampin (RMP) and desacetyl RMP (DRMP) *(B),* and isoniazid (INH) and acetyl INH (AcINH) *(C)* excreted in urine up to 8 h after dosing in patients with tuberculosis (TB), HIV infection and diarrhea (HIV), and HIV and TB (HIVTB). *Vertical bars,* SD. *$P < .05$.

gastrointestinal disorders and AIDS have less predictable absorption of antimycobacterial drugs. Our study provides a qualitative estimate of the effect of HIV infection on the absorption of the 2 key anti-TB drugs (rifampin and isoniazid) based on the urinary excretion of the drugs and their metabolites.

It has been reported that urine kinetics are as good a means as blood kinetics to obtain information on the bioavailability of anti-TB drugs [15–17]. Because the procedure is noninvasive and relatively easy to perform, obtaining estimates of urine excretion of rifampin and isoniazid and their metabolites was undertaken in this study. Creatinine levels were determined in all urine samples to ensure that the urine collections were complete (i.e., the entire volume of urine excreted by the patient during the study period was collected).

The data obtained in this study clearly demonstrate that the rate of urinary excretion of both rifampin and isoniazid was significantly lower in both groups of HIV-infected patients than in the TB group. These findings are supported by a significant decrease in D-xylose absorption as well.

There does not appear to be a single patient characteristic or diagnostic test that reliably predicts the propensity for drug malabsorption in an individual patient, although a low CD4 count and gastrointestinal disturbances appear to increase the likelihood of significant malabsorption [8]. This aspect has been confirmed by our data, because we found a significant correlation between CD4 lymphocyte count and urinary level of rifampin. The results of this study further suggest that D-xylose absorption testing of urine samples, which is simple to perform, can be used to screen HIV-infected patients who may not be absorbing rifampin and isoniazid adequately. This observation is in agreement with the report by Sahai et al. [8]. Among the 26 HIV-infected patients with TB, 11 had chronic diarrhea. However, diarrhea did not seem to influence the absorption of rifampin and isoniazid, as evidenced by the fact that HIV-infected patients without diarrhoea did not differ significantly from patients with diarrhea with regard to urinary levels of D-xylose, rifampin, and isoniazid.

Our preliminary findings are, to our knowledge, the first on the existence of malabsorption of anti-TB drugs in HIV-infected patients from the Indian subcontinent. We have documented the presence of a significant degree of malabsorption among patients with advanced HIV infection, with and without diarrhea. This could have implications for treatment of TB in HIV-infected patients. A larger prospective study would be required to correlate low drug levels and poor response to anti-TB treatment. The population in this study included both patients with new and patients with previously treated TB and were generally in an advanced stage of HIV disease. Clinicians must maintain a high index of suspicion for drug malabsorption in patients with AIDS. Some authors recommend therapeutic drug monitoring of anti-TB drugs in patients with AIDS

observations that standard multiple-drug regimens are as effective in HIV-infected patients as in HIV-uninfected patients, there have been disconcerting reports of low levels of anti-TB drugs in patients with HIV infection and TB [2–6, 8].

Most patients with TB but without other illnesses absorb antimycobacterial drugs reliably [14]. This finding is in agreement with ours: the excretion of D-xylose was similar in patients with TB and healthy volunteers. However, patients with

[18]. This proposal has significant cost and health policy implications. Alternately, estimation of rifampin and isoniazid levels in urine, as was done in this study, could provide important information on the absorption of these drugs. Pharmacokinetic studies are in progress to confirm the results obtained in this study.

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