Indian J Med Res 125, June 2007, pp 763-766

Urine levels of rifampicin & isoniazid in asymptomatic HIV-positive individuals

Geetha Ramachandran, A.K. Hemanth Kumar, K. Sarala*, C. Padmapriyadarsini, S. Anitha, C.B. Tharani*, V. Kumaraswami & Soumya Swaminathan

*Tuberculosis Research Centre (ICMR), Chennai, *Department of Pharmacology Madras Medical College, Chennai, India*

Received January 12, 2006

***Background & objectives*: AIDS and its associated gastrointestinal complications may impair the absorption of anti-tuberculosis (TB) drugs. Impaired absorption of anti-TB drugs could lead to low drug exposure, which might contribute to acquired drug resistance and reduced effectiveness of anti-TB treatment. The aim of this study was to obtain information on the status of absorption of rifampicin (RMP) and isoniazid (INH) in asymptomatic HIV- positive individuals, who are less immunocompromised. The D-xylose absorption test was also carried out to assess the absorptive capacity of intestive.**

***Methods*: The absorption of RMP, INH and D-xylose was studied in 15 asymptomatic HIV- positive individuals with CD4 cell counts > 350 cells/mm^3 and 16 healthy volunteers, after oral administration of single doses of RMP (450 mg), INH (300 mg) and D-xylose (5 g). Urine was collected up to 8 h after drug administration. Percentage dose of the drugs and their metabolites and D-xylose excreted in urine were calculated.**

***Results*: A significant reduction in the urinary excretion of INH and D-xylose in HIV-positive persons compared to healthy volunteers was observed. The per cent dose of RMP and its metabolite, desacetyl RMP was also lower in HIV-positive persons compared to healthy volunteers, but this difference was not statistically significant.**

***Interpretation & conclusion*: Decreased urinary excretion of D-xylose and INH are suggestive of intestinal malabsorption in HIV-positive individuals. HIV infection could cause malabsorption of anti-TB drugs even at an early stage of the disease. The clinical implications of these findings need to be confirmed in larger studies.**

Key words Asymptomatic HIV infection - isoniazid - malabsorption - rifampicin

Response to rifampicin (RMP) - based antimycobacterial therapy is generally good in HIV-infected patients with tuberculosis (TB)[1]. It has, however, been widely reported that AIDS and its associated gastrointestinal complications may impair the absorption of anti-TB drugs. Decreased absorption of anti-TB drugs in an HIV-infected patient was reported as early as 1992[2]. Since that time, several investigators have documented poor bioavailability of anti-TB drugs in HIV-infected patients with and without TB[3-7]. Others have reported normal absorption in HIV infection[8-10]. Impaired absorption of anti-TB drugs could lead to low drug exposure, which might contribute to acquired drug resistance and reduced effectiveness of anti-TB treatment[11]. A significant correlation between the degree of immune suppression and malabsorption has been

reported by Keating *et al*[12]. We have earlier reported a significant degree of malabsorption of anti-TB drugs among patients with advanced HIV disease, with and without diarrhoea. These findings were based on both blood[5] and urine[4] levels of anti-TB drugs. We extended our study to obtain information on the status of absorption of RMP and isoniazid (INH) in asymptomatic HIV-positive individuals, who are less immunocompromised, by measuring the urinary excretion of these drugs. The D-xylose absorption test to assess the absorptive capacity of the intestines was also performed.

Material & Methods

Participants: Sixteen healthy volunteers, willing staff members working at Tuberculosis Research Centre, Chennai and not known to be seropositive for HIV infection and 15 HIV seropositive asymptomatic persons participated in the study. Eligible HIV-positive individuals were selected from those attending the outpatient clinic of the Tuberculosis Research Centre, between June to September 2005. Asymptomatic persons were classified as having stage I A or II A infection as defined by the Centers for Disease Control and Prevention classification system, 1993[13]. The sample size was chosen based on the study findings reported by Agarwal *et al*[14]. All study participants were males and were required to meet the following criteria: *(i)* age 18-50 yr, *(ii)* no significant hepatic or renal dysfunction (liver transaminases, blood urea and creatinine within normal limits), *(iii)* non-diabetic, *(iv)* CD4 cell counts ≥ 350 cells/μl^3, *(v)* not taken RMP or INH for atleast a month before start of the study, and *(vi)* willing to give informed written consent. Diagnosis of HIV infection was based on 3 positive results (2 rapid tests, Tridot, Mitra and Co. and Combaids, Span Diagnostics, India followed by ELISA, Labsystems). None of the HIV seropositive persons was receiving antiretroviral treatment. The study was conducted after obtaining approval from the institutional ethics committee, and informed written consent was obtained from all the study participants before they took part in the study.

Conduct of study: Baseline demographic, clinical and laboratory data were obtained from all eligible study participants. They were asked to report to the clinic in the morning after an overnight fast. They were instructed to empty their bladder and then received RMP (450 mg) and INH (300 mg) orally under supervision. The exact time of drug administration was noted. Two hours later, a uniform oral dose of D-xylose (5 g) in water was administered. Urine excreted up to 8 h after drug administration was collected in labeled containers. The study was conducted under the complete supervision of the study investigators. Care was taken to ensure that the urine collections were complete. The volume of urine was measured and aliquots were stored at -20°C. Ascorbic acid was added to urine aliquots to prevent oxidation of RMP.

Determination of INH acetylator status: The INH acetylator status of all the study participants was determined by differentially estimating the concentrations of INH and its primary metabolite, acetyl INH (AINH) in urine excreted between 5 and 6 h after oral administration of 300 mg INH, and by calculating the molar ratio of AINH to INH. The acetylator status was considered to be rapid when the ratio was 2.0 or more[15].

Drug estimations: The concentrations of RMP and its primary metabolite, desacetyl RMP (DRMP) were measured by high performance liquid chromatography (HPLC)[16]. The concentrations of INH and AINH[17] and that of D-xylose[18] were measured by spectrophotometric methods. The values were expressed as percentage dose of RMP (RMP & DRMP), INH (INH & AINH) and D-xylose excreted in urine.

Statistical analysis: Analysis of data was performed using SPSS software package, version 13.0. The mean percentage doses of D-xylose, RMP and INH excreted in urine were compared between HIV-positive individuals and healthy volunteers by independent t-test. Significance was taken at the 5 per cent level.

Results

There were no significant differences in the mean age and body weight between HIV-positive individuals and healthy volunteers (Table I). The mean per cent doses of D-xylose, and INH and AINH excreted in urine were significantly lower in HIV-positive individuals compared to healthy volunteers (Table II). The mean per cent doses of RMP and DRMP excreted in urine of healthy volunteers and HIV-infected individuals were 10.8 and 8.0 per cent respectively, the difference was not statistically significant. The per cent dose of INH excreted in urine was calculated, and comparisons were made between HIV-positive individuals and healthy volunteers among slow and rapid acetylators separately. The mean per cent doses of INH among slow acetylators were 29.6±1.9 per cent in healthy volunteers and 25.5±3.7 per cent in HIV-positive individuals ($P<0.05$). The corresponding values among rapid acetylators were 15.2±3.0 per cent and 11.5±3.8 per cent respectively. The decrease in urinary excretion of INH in HIV-positive persons compared to healthy volunteers among slow and rapid acetylators was 14 and 24 per cent respectively.

Discussion

All the HIV-positive individuals who took part in the study had CD4 cell counts greater than or equal to 350 cells/mm^3. Although they were not immunocompromised, the percentage doses of RMP and INH excreted in urine were decreased in asymptomatic HIV-positive individuals when compared to healthy volunteers. This observation points to the fact that HIV infection causes malabsorption of anti-TB drugs even at an early stage of the disease. This is supported by a study carried out by Knox *et al*[19] who found impaired absorptive function in 88 per cent of 671 HIV-infected persons studied. They concluded that gastrointestinal dysfunction was common among HIV-positive persons, appeared early in the course of infection, in the absence of diarrhoea and in persons with CD4 counts >200 cells/mm^3.

Our findings are in agreement with that reported by Sahai *et al*[3], who observed reduction in exposure and peak concentration of certain anti-TB drugs in asymptomatic HIV-positive individuals. They observed a significant and systematic decrease in blood levels of certain anti-TB drugs when studying healthy volunteers at one end of the spectrum to symptomatic HIV patients with diarrhoea at the other end of the spectrum. When our present data, obtained in asymptomatic HIV-positive individuals, were compared with that obtained from our earlier study[4] done in symptomatic HIV patients with and without TB, it was found that the percentage doses of D-xylose, RMP and INH obtained in this study were between pulmonary TB patients (control group) and patients with advanced HIV disease. This suggests that there is a trend of decreased absorption and urinary excretion of D-xylose, RMP and INH occurring in HIV-infected persons as their disease advances, similar to the observations made by Sahai *et al*[3]. Keating *et al*[12], however, did not observe malabsorption of D-xylose in asymptomatic HIV-infected persons, but found increased intestinal permeability in all sub-groups of patients, and a significant correlation between malabsorption and degree of immunosuppression. Our earlier observations[20,21] and that of others[22, 23] have pointed to the fact that bioavailability indices of anti-TB drugs, calculated based on blood and urine levels are similar. Urine estimations have the added advantage of being non-invasive and easy to perform. However, care must be taken to ensure that the urine collections are complete within the stipulated time periods.

The metabolizing enzyme of INH is a hepatic N-acetyl transferase, which displays genetic polymorphism. The difference in the two phenotypes of this enzyme, namely, slow and rapid acetylators of INH is due to difference in quantity rather than quality of the enzyme, the rapid acetylators having 4-5 times the quantity of the enzyme as the slow acetylators. It is therefore expected that rapid acetylators will have enhanced metabolism of INH, and that blood levels and urinary excretion of INH will be lower in rapid than in slow acetylators. This observation was confirmed in our study.

In conclusion, this study demonstrates that HIV infection, regardless of the stage of the disease may lead to malabsorption of anti-TB drugs. Clinicians caring for HIV-positive patients may need to consider assessing malabsorption in patients with inadequate response/ failure/development of drug resistance. Urine levels of anti-TB drugs can be monitored in HIV-infected patients. This is particularly important in those who are slow to respond to therapy. These findings are also important because many individuals with HIV infection are started on preventive therapy for TB with INH or a combination of two or three anti-TB drugs. One of the reasons for

Table I. Characteristics of study participants and healthy volunteers

Characteristic	Group	
	Healthy volunteers (n = 16)	Asymptomatic HIV-positive individuals (n = 15)
Mean age ±SD (yr)	36 ± 9.9(23-52)	32 ± 6.7 (25-50)
Mean body weight ±SD (kg)	63 ± 8.5(48-76)	56 ± 7.5(41-71)
Median CD4 cell count (cells/mm^3)		425 (350-853)
INH acetylator status (No. of participants)		
Slow	8	8
Rapid	8	7

Values are mean ± SD
Ranges are given in parentheses

Table II. Percentage dose (mean ± SD) of D-xylose, rifampicin and isoniazid excreted in urine in different study groups

	D-Xylose	RMP + DRMP	INH + AINH
Healthy volunteers (n=16)	29.3 ± 4.6	10.8 ± 5.5	46.0 ± 4.2
HIV-positive individuals (n = 15)	23.1** ± 5.4	8.0 ± 2.0	40.1* ± 8.3

P * <0.05 **<0.01 compared to healthy volunteers
DRMP, desacetyl RMP
AINH, acetyl INH

failure of preventive therapy may be malabsorption and inadequate blood levels of anti-TB drugs. Further studies are required to correlate drug levels and clinical outcomes both in the treatment and prevention of TB in HIV-positive individuals.

Acknowledgment

The authors thank Dr P.R. Narayanan, Director, Tuberculosis Research Centre, Chennai, India for his encouragement and support, Shri B. Doraiswamy for secretarial assistance, and all the patients and healthy volunteers who took part in the study.

References

1. Small PM, Schecter GF, Goodman PC, Sande MA, Chaisson RE, Hopewell PC. Treatment of tuberculosis in patients with advanced human immunodeficiency virus infection. *N Engl J Med* 1991; *324* : 289-94.
2. Berning SE, Huitt GA, Iseman MD, Peloquin CA. Malabsorption of anti-tuberculosis medications by a patient with AIDS. *N Engl J Med* 1992; *327* : 1817-8.
3. Sahai J, Gallicano K, Swick L, Tailor S, Garber I, Seguin L, *et al*. Reduced plasma concentrations of anti-tuberculosis drugs in patients with HIV infection. *Ann Intern Med* 1997; *127* : 289-93.
4. Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Padmapriyadarsini C, Swaminathan S, *et al*. Malabsorption of rifampicin and isoniazid in HIV-infected patients with and without tuberculosis. *Clin Infect Dis* 2004; *38* : 280-3.
5. Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Padmapriyadarsini C, Swaminathan S, *et al*. Decreased bioavailability of rifampin and other anti-tuberculosis drugs in patients with advanced human immunodeficiency virus disease. *Antimicrob Agents Chemother* 2004; *48* : 4473-5.
6. Peloquin CA, MacPhee AA, Berning SE. Malabsorption of antimycobacterial medications. *N Engl J Med* 1993; *329* : 1122-3.
7. Peloquin CA, Nitta AT, Burman WJ, Brudney KF, Miranda-Massari JR, McGuinnes ME, *et al*. Low antituberculosis drug concentrations in patients with AIDS. *Ann Pharmacother* 1996; *30* : 919-23.
8. Taylor B, Smith PJ. Does AIDS impair the absorption of anti-tuberculosis agents? *Int J Tuberc Lung Dis* 1998; *2* : 670-5.
9. Conte JE, Golden JA, McQuitty M, Kipps J, Duncan S, McKenna E, *et al*. Effects of gender, AIDS and acetylator status on intrapulmonary concentrations of isoniazid. *Antimicrob Agents Chemother* 2002; *46* : 2358-64.
10. Jaruratanasirikul S. The pharmacokinetics of oral rifampicin in AIDS patients. *J Med Assoc Thai* 1998; *81* : 25-8.
11. Alwood K, Keruly J, Moore-Rice K, Stanton DL, Chaulk PC, Chaisson RE. Effectiveness of supervised intermittent therapy for tuberculosis in HIV-infected patients. *AIDS* 1994; *8* : 1103-8.
12. Keating J, Bjarnason I, Somasundaram S, Macpherson A, Francis N, Price AB, *et al*. Intestinal absorptive capacity, intestinal permeability and jejunal histology in HIV and their relation to diarrhea. *Gut* 1995; *37* : 623-9.
13. Bartlett JG, Gallant JE. Medical management of HIV infection. Baltimore: John Hopkins University School of Medicine, 2004. Available at: *www.hopkins-hivguide.org*.
14. Agrawal S, Kaur KJ, Singh I, Bhade SR, Kaul CL, Panchagnula R. Determination of rifampicin bioequivalence in a three - drug FC by WHO and Indian protocols : effect of sampling schedule and size. *Int J Tuberc Lung Dis* 2005; *9* : 75-80.
15 Sharma GR, Kannapiran M, Narayana ASL, Radhakrishna S, Tripathy SP. Determination of acetylator phenotype based on the ratio of acetyl isoniazid to isoniazid in urine following an oral dose of ordinary isoniazid. *Indian J Med Res* 1976; *64* : 1-8.
16 Hemanth Kumar AK, Immanuel C, Ramachandran G, Silambuchelvi K, Victor L, Gurumurthy P. A validated high-performance liquid chromatography method for the determination of rifampicin and desacetyl rifampicin in plasma and urine. *Indian J Pharmacol* 2004; *36* : 231-3.
17. Rao KVN, Kailasam S, Menon NK, Radhakrishna S. Inactivation of isoniazid by condensation in a syrup preparation. *Bull World Health Orgn* 1971; *45* : 625-32.
18. Tietz NW. Gastric, pancreatic and intestinal function. In: Burtis CA, Ashwood ER, editors. *Fundamentals of clinical chemistry*. Philadelphia: WB Saunders; 1976 p. 1063-99.
19. Knox TA, Spiegelman D, Skinner SC, Gorbach S. Diarrhea and abnormalities of gastrointestinal function in a cohort of men and women with HIV infection. *Am J Gastroenterol* 2000; *95* : 3482-9.
20. Gurumurthy P, Ramachandran G, Vijayalakshmi S, Hemanth Kumar AK, Venkatesan P, Chandrasekaran V, *et al*. Bioavailability of rifampicin, isoniazid and pyrazinamide in a triple drug formulation: comparison of plasma and urine kinetics. *Int J Tuberc Lung Dis* 1999; *3* : 119-25.
21. Immanuel C, Gurumurthy P, Ramachandran G, Venkatesan P, Chandrasekaran V, Prabhakar R. Bioavailability of rifampicin following concomitant administration of ethambutol or isoniazid or pyrazinamide or a combination of the three drugs. *Indian J Med Res* 2003; *118* : 109-14.
22. Panchagnula R, Kaur KJ, Singh I, Kaul CL. The WHO simplified study protocol in practice: investigation of combined formulations supplied by the WHO. *Int J Tuberc Lung Dis* 1999; *3* (Suppl) : S336-42.
23. Pillai G, Ellard GA, Smith PJ, Fourie PB. The potential use of urinary excretion data for assessing the relative bioavailability of rifampicin in fixed dose combination of anti-tuberculosis formulations. *Int J Tuberc Lung Dis* 2001; *5* : 691-5.

Reprint requests: Dr Soumya Swaminathan, Deputy Director (Sr. Gr.) & Head, HIV/AIDS Division, Tuberculosis Research Centre
Mayor V.R. Ramanathan Road, Chetput, Chennai 600031, India
e-mail: doctorsoumya@yahoo.com