Acetylator status influences bioavailability of isoniazid in patients with advanced HIV disease

Running Head: Bioavailability of isoniazid in HIV disease

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Abstract

Patients with advanced HIV disease may exhibit malabsorption of anti-tuberculosis(TB) drugs. We evaluated the effect of isoniazid (INH) acetylator status on the bioavailability of INH in HIV-infected patients with and without tuberculosis, based on urinary excretion of the drug. Estimation of INH in urine collected up to 8 hours after oral administration of 300 mg INH were undertaken in 23 TB, 40 HIV and 26 HIV-TB patients. Determination of acetylator status of all these patients was also carried by differential estimations of INH and acetyl INH in urine collected between 5 and 6 hours after oral administration of 300 mg INH.

Both slow and rapid acetylators in HIV and HIV-TB groups had significantly lower concentration of INH in urine compared to TB patients. The percent decrease in urinary excretion of INH was significantly higher in rapid than in slow acetylators, when compared to the corresponding TB patients. Acetylator status has an impact on the bioavailability of INH. Malbsorption in patients with advanced HIV disease may lead to decreased bioavailability of INH, particularly in rapid acetylators. Urinary estimation of INH provides reliable information on the bioavailability of the drug.

Key words: Acetylator status**,** Bioavailability, Isoniazid, HIV, Urine

Introduction

Isoniazid (INH) is an essential drug in the treatment of tuberculosis (TB). The primary step in its metabolism is acetylation to acetyl INH. The metabolising enzyme is a hepatic N-acetyl transferase, which displays genetic polymorphism. The rate of acetylation of INH is known to influence the response to treatment of TB patients with once-weekly regimens containing INH[1,2] but it is of no prognostic significance when patients are treated with either daily, thrice-weekly or twice-weekly regimens containing the drug. It was observed that the failure of once-weekly regimens was predominantly due to inadequate exposure (area under the time concentration curve) and coverage (hours for which a bacteriostatic concentration of INH 0.2 µg/ml are maintained)[3]. Weiner et al[4] have reported that once-weekly INH / rifapentine therapy was less effective than twice-weekly INH / rifampicin in HIV sero negative TB patients. They observed an association between INH acetylator status and treatment outcome in these patients and further found that low INH concentrations in rapid acetylators was associated with failure/relapse. It is therefore essential that adequate INH concentrations are maintained in blood for good treatment outcome.

In a recent pharmacokinetic study conducted in HIV-infected patients with advanced disease, we observed that peak concentration and exposure of INH were reduced in HIV-infected patients with and without TB compared to HIV seronegative pulmonary TB patients[5]. This study further showed that the differences in these pharmacokinetic variables were more pronounced in rapid acetylators than in slow acetylators of INH. However,

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these differences (except for peak concentration in HIV-TB rapid group) failed to attain statistical significance. This could probably be due to the small number of patients in each sub-group (after classifying them based on their acetylator status). The study suggested that acetylator status could influence the bioavailability of INH in HIV-infected patients, and that rapid acetylators are likely to be affected more than slow acetylators, in the event of malabsorption.

Several reports suggest that malabsorption of anti-mycobacterial drugs occurs in selected HIV-infected patients, particularly those with advanced disease[5-12]. This could lead to decreased blood levels of anti-TB drugs, thereby causing acquired drug resistance and treatment failure/relapse. The objective of the present study was to evaluate the effect of INH acetylator status on the bioavailability of INH in HIV-infected patients with and without TB, based on the urinary excretion of the drug. For this purpose, we carried out estimation of INH in urine and determination of INH acetylator status in urine samples collected from patients who participated in a previous study[12].

Methods

Participants

The study was conducted at the Government Hospital of Thoracic Medicine, Chennai, India. The study participants comprised of 23 HIV seronegative patients with pulmonary TB, 40 patients with advanced HIV disease and diarrhoea (history of recurrent episodes of watery stools, six to eight times a day for at least 10 consecutive days in a month at the start of study) and 26 patients with HIV infection and TB. The mean age and mean body weight of TB, HIV and HIV-TB patients were 41, 34 & 37 years and 40, 43 & 43 kg respectively. All the participants were males and were admitted to the hospital at least a day prior to the study. None of the patients was suffering from significant hepatic or renal dysfunction (liver transaminases, serum urea and creatinine were within normal limits). Diagnosis of pulmonary TB was based on bacteriological investigations (sputum smear and culture for mycobacteria), which were supported by clinical and radiological features. HIV infection was diagnosed on the basis of three positive results (two rapid tests and ELISA). All TB patients, with and without HIV infection were receiving standard anti-TB regimens. None of the HIV-infected patients were receiving antiretroviral treatment or any stool binders or other medications known to interfere with the absorption of INH. The study was conducted after obtaining approval from the Institutional Ethics Committee and informed written consent was obtained from all the study participants.

Conduct of study

Isoniazid was withheld for a period of 72 hours before start of the study. All the patients were asked to empty their urinary bladder and this urine sample was discarded. They received INH (300mg) orally under supervision after an overnight fast. In addition to this, TB and HIV-TB received their other regular anti-TB medications. Uniform breakfast and lunch were provided to all the patients at 2 and 6 hours after drug administration. Urine excreted up to 8 hours was collected in a labeled container. The total volume of urine was measured, volume noted and aliquots were stored at -20^{o}C until analysis. The concentration of INH in all the urine aliquots was estimated by a spectrophotometric method[13]. The percent dose of INH excreted in urine was calculated. All estimations were undertaken after coding the samples.

Determination of INH acetylator status

The INH acetylator status of all the patients was determined by differentially estimating the concentration of INH and its primary metabolite, acetyl INH in urine excreted between 5 and 6 hours after oral administration of 300 mg INH, and by calculating the molar ratio of acetyl INH to INH. A ratio of 2.0 or more denoted the acetylator status to be rapid[14.]

Statistical analysis

Analysis of data was performed using SPSS (version 10.5) package. The significance of differences in mean percent dose between two study groups was evaluated using unpaired t-test and between more than two groups using Bonferoni multiple comparison test (one-way Anova). The significance was taken at 5% level.

Results and Discussion

The number of slow and rapid acetylators of INH among TB, HIV and HIV-TB patients were 16 & 7, 22 & 18 and 11 & 15 respectively. The percent dose of INH excreted in urine up to 8 hours was compared between HIV (with and without TB) and TB patients among slow and rapid acetylators independently. Likewise, the percent

decrease in INH excretion in HIV and HIV-TB patients was calculated separately for slow and rapid acetylators against the corresponding TB patients in both these groups. There was a significant decrease in percent dose of INH excreted in urine in HIV and HIV-TB patients when compared to TB patients in both slow and rapid acetylators of INH ($p<0.01$) (Table 1). While the percent decrease in urinary excretion of INH in slow acetylators in HIV and HIV-TB patients against TB patients was 28% and 27% respectively, the corresponding values among rapid acetylators was 47% and 55% respectively. The difference in urinary INH values between HIV and HIV-TB patients was not statistically significant.

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 than slow acetylators, and that blood levels and urinary excretion of INH will be lower in rapid than in slow acetylators. A significant effect of acetylator status on blood levels of INH has been reported[4, 5, 11, 15]. This aspect was further confirmed in this study, where a significant decrease in urinary excretion of INH in rapid compared to slow acetylators in all three groups of patients was observed. This study further demonstrated that the percent decrease in INH excretion in HIV and HIV-TB patients was higher in rapid than in slow acetylators.

In the Revised National TB Control Programme, where INH is administered thrice-weekly, INH acetylator status may not play a role in treatment outcome as demonstrated in certain studies[1, 2]. Despite a 50% reduction in the percent dose of INH excreted in urine in rapid than in slow acetylators among TB patients, as seen in this study, the treatment outcome is expected to be similar irrespective of their INH acetylator status. However, the presence of malabsorption in rapid acetylators with advanced HIV disease (with and without TB) may lead to decreased bioavailability of INH. This could have implications for treatment of TB in a subset of HIV-infected patients who may require higher dose of INH or daily treatment. On the other hand slow acetylators are less likely to be affected, since their urinary INH levels were higher than TB patients who were rapid acetylators. This has been confirmed by our earlier study, where we observed differences in peak concentration and exposure between TB and HIV patients to be significant only among rapid acetylators of INH[5].

To the best of our knowledge, there are no reports available on the effect of INH acetylator status on the bioavailability of INH in HIV-infected patients. Our findings suggest that patients with HIV, TB, diarrhoea who are rapid acetylators might have reduced bioavailability of INH. Urinary INH estimations offer a simple and reliable non invasive procedure to obtain information on the bioavailability of INH. A large prospective trial is required to correlate INH acetylator status with treatment outcome and emergence of mycobacterial drug resistance in patients with advanced HIV disease.

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Table 1 Urinary excretion of INH in the different study groups

	Percent dose of INH (Mean ± SD)		
INH acetylator status	TB(S-16; R-7)	HIV(S-22; R-18)	HIV-TB(S-11; R-15)
Slow	31.71±7.98	22.80*±7.19 (28%)	23.28*±4.24 (27%)
Rapid	15.83±2.13	8.38*±3.62 (47%)	7.12*±3.02 (55%)

*p< 0.01 (vs TB patients)
Percent decrease vs TB patients are given in parentheses
S = Slow acetylator of INH, R = Rapid acetylator of INH

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