

Lack of association between plasma levels of non-nucleoside reverse transcriptase inhibitors & virological outcomes during rifampicin co-administration in HIV-infected TB patients

Geetha Ramachandran, A.K. Hemanth Kumar, C. Ponnuraja, K. Ramesh, Lakshmi Rajesh, C. Chandrasekharan* & Soumya Swaminathan

*National Institute for Research in Tuberculosis (ICMR) & *Government Hospital of Thoracic Medicine, Chennai, India*

Received November 9, 2011

Background & objectives: Among patients with HIV-associated tuberculosis (TB), reduced plasma non-nucleoside reverse transcriptase inhibitors (NNRTI) concentrations during rifampicin (RMP) co-administration could lead to HIV treatment failure. This study was undertaken to examine the association between plasma nevirapine (NVP) and efavirenz (EFV) concentrations and virological outcomes in patients infected with HIV-1 and TB.

Methods: This was a nested study undertaken in a clinical trial of patients with HIV-1 and TB, randomized to two different once-daily antiretroviral treatment (ART) regimens along with anti-TB treatment (ATT). Trough concentrations of plasma NVP and EFV were estimated at months 1 (during ATT and ART) and 6 months (ART only) by HPLC. Plasma HIV-1 RNA level >400 copies/ml or death within 6 months of ART were considered as unfavourable outcomes. Genotyping of *CYP2B6* 516G>T polymorphism was performed.

Results: Twenty nine per cent of patients in NVP arm had an unfavourable outcome at 6 months compared to 9 per cent in EFV arm ($P<0.08$). The mean NVP and EFV levels estimated at 1 and 6 months did not significantly differ between favourable and unfavourable responders. Logistic regression analysis showed *CYP2B6* 516G>T polymorphism significantly associated with virologic outcome in patients receiving EFV-based regimen.

Interpretation & conclusions: Trough plasma concentrations of NVP and EFV did not show any association with response to ART in patients on ATT and once-daily ART. *CYP2B6* 516G>T polymorphism was associated with virologic outcome among patients on EFV.

Key words HIV-1 & tuberculosis - NNRTIs - rifampicin - TDM - virologic outcome

The non-nucleoside reverse transcriptase inhibitors (NNRTI), efavirenz (EFV) and nevirapine (NVP) are essential components of life-saving antiretroviral treatment (ART) for HIV infection in resource-limited settings. Tuberculosis (TB) is the most common complication of HIV infection and is associated with high fatality rates¹. Rifampicin (RMP), a key component of anti-TB regimens induces the expression and activity of the cytochrome P450 (CYP) metabolic enzymes in the liver². The drug-drug interactions between RMP and the NNRTIs result in the reduction in NNRTI concentrations which may potentially lead to HIV treatment failure and development of drug resistance in HIV infected TB patients. The effect of RMP on blood NVP level is greater than on EFV level³.

It has been reported that plasma NNRTI concentrations below the lower limit of the therapeutic range increase the risk of development of HIV drug resistance due to incomplete virological suppression⁴, while levels above the upper limit increase the risk of drug-related toxicity⁵. Monitoring plasma NNRTI concentrations in HIV-TB patients, especially during anti-TB treatment (ATT) with RMP may be a useful tool to ensure that the drug levels remain within the therapeutic range. Our group conducted a randomized controlled clinical trial to compare the efficacy and safety of a once-daily antiretroviral regimen of didanosine, lamivudine and NVP versus a standard regimen of didanosine, lamivudine and EFV among patients with HIV-1 infection and TB⁶. A sub-study was planned to estimate plasma concentrations of NVP and EFV during and after completion of ATT in patients who were enrolled in this trial with the aim of examining the association between plasma NVP and EFV concentrations and virological outcomes, and to assess if therapeutic drug monitoring (TDM) is a useful strategy.

Material & Methods

This was a prospective study undertaken in patients with HIV-1 and TB, who were enrolled in a randomized controlled clinical trial at the Tuberculosis Research Centre (now National Institute for Research in Tuberculosis), Chennai, India (NCT#00332306) during May 2006 to June 2008. All patients were adults, naïve to ART, newly diagnosed with active TB, non-pregnant and had CD4+ cell counts <250 cells/ μ l. Exclusion criteria included previous ATT or ART for >1 month, HIV-2 infection, major psychiatric illness, aspartate aminotransferase and alanine aminotransferase levels >2.5 times the upper limit of normal and having a severe

non-HIV related disease. Pulmonary TB was diagnosed based on sputum smear and culture examination. Three sputum specimens (1 spot and 2 overnight samples) were collected and smears were stained with auramine-rhodamine and examined by fluorescent microscopy; sputum was processed by modified Petroff's method and cultured on Lowenstein-Jensen (L-J) medium^{7,8}. Extra-pulmonary TB was diagnosed based on cyto- and histopathological (for lymph node specimens) or radiographic and biochemical (for pleural effusion specimens) parameters. The biochemical parameters tested in the pleural effusion for diagnosing TB were differential cell count, protein and lactate dehydrogenase (LDH). The fluid to plasma ratio was calculated to determine if the effusion was a transudate or an exudate. A fluid to plasma protein ratio of >0.5, fluid to plasma LDH ratio of >0.6 and a lymphocytic predominance of the fluid were taken as evidence of probable TB effusion. The Institutional Ethics Committee approved the study protocol, and all patients gave informed written consent before enrollment.

All patients received the standard national anti-TB regimen with RMP (450 mg for body weight <60 kg and 600 mg for body weight \geq 60 kg), isoniazid (600 mg), pyrazinamide (1500 mg) and ethambutol (1200 mg) thrice weekly for the first two months, followed by RMP and isoniazid at the same doses thrice weekly for the subsequent four months. After the intensive phase of ATT, patients were randomized to receive either of the once-daily antiretroviral regimens along with the continuation phase of ATT. The ART regimen consisted of lamivudine (300 mg) and didanosine (250 mg for body weight <60 kg and 400 mg for body weight \geq 60 kg) with either NVP (400 mg) or EFV (600 mg) once daily. NVP was started at 200 mg once-daily for the first two weeks. All drugs were administered under direct observation in the clinic three days a week and supplied to the patient for the remaining days. In addition to ATT and ART, all patients received one tablet of trimethoprim-sulfamethoxazole at double strength, pyridoxine 10mg, and multivitamins daily, in combination with psychosocial and adherence counseling.

Permuted block randomization (blocks of 8) was done centrally using a computer-generated list of random numbers, stratified by site and two levels of CD4+ cell count (\leq 150 vs >150 cells/ μ l) and viral load (\leq 50,000 vs >50,000 copies/ml) each.

Measurement of CD4 cell counts by flow cytometry (Becton, Dickinson FACSCount flow cytometer, USA)

and HIV-1 RNA levels using COBAS® AMPLICOR HIV-1 Monitor Test, v1.5 (Roche Molecular Systems, USA) were performed at baseline, two and six months. Plasma HIV-1 RNA level >400 copies/ml at six months of ART (confirmed by repeat measurement) and death within 6 months (in an intention-to-treat analysis) were considered as unfavourable responses.

Estimation of plasma NVP and EFV was performed at two time points, *i.e.* at one and six months after initiation of ART. Month one corresponded to the time when patients had received three months of ATT and one month of ART, and month six to the period when patients had completed ATT and were receiving only ART. At months one and six, two ml blood was collected before drug administration corresponding to trough concentration. Plasma NVP and EFV levels were estimated by HPLC according to validated methods^{9,10}. A small portion of the blood sample was used for genotyping of *CYP2B6* 516G>T and 983 T>C polymorphisms. Genomic DNA extracted was from whole blood and the *CYP2B6* amplicon directly sequenced using 3100 Avant Genetic analyzer¹¹. The *CYP2B6* 983 T>C polymorphism was genotyped using Real-time PCR¹².

To calculate the sample size we assumed a favourable virologic response rate of 90 per cent at 24 wk with the EFV regimen and a noninferiority margin of 15 per cent for the NVP regimen. With a power of 80 per cent and a significance level of 5 per cent, the sample size per group was calculated as 90, allowing for a 20 per cent loss due to death or loss to follow up. However, the Data Safety and Monitoring Board withheld intake to the study after 116 patients had been randomized.

Statistical analysis: Analysis of data was performed using SPSS, version 14.0 (SPSS Inc. Chicago, IL, USA), and data expressed as mean \pm SD after normality was checked using Kolmogorov Smirnov and Shapiro Wilk tests. Comparisons of plasma concentrations of NVP and EFV between patients with favourable and unfavourable response to ART, and between those on and off RMP were done using t-test. Chi-squared test was performed to compare the proportion of patients with sub-therapeutic NVP/EFV levels among favourable and unfavourable responders. In this study, plasma NVP and EFV concentrations below 3.4 and 1.0 $\mu\text{g/ml}$, respectively were taken as sub-therapeutic¹³. Group-wise comparison of plasma concentrations among the different genotypes of *CYP2B6* 516G>T was done using Tukey's multiple comparison test.

Logistic regression analysis by backward elimination method was carried out to identify those variables that were significantly associated with virologic failure.

Results

A total of 107 patients, 52 and 55 in the NVP and EFV arms, respectively were included in this sub-study. At baseline, the two groups had similar demographic and clinical characteristics, except for haemoglobin which was significantly different between the two groups ($P=0.041$) (Table I). There was a significant increase in body mass index, hemoglobin and CD4+ cell counts between baseline and 6 months in both groups ($P<0.001$). Ten patients in the NVP arm and five in the EFV arm had viral load >400 copies/ml at six months ($P<0.001$). The CD4 cell counts in these patients at 6 months were 261 ± 152 and 247 ± 111 cells/ μl , respectively.

Among the 52 in the NVP arm, 15 (28.8%) had an unfavourable response; 10 had viral load >400 copies/ml at 6 months while five had died. Among the 55 patients in the EFV arm, five (9.1%) had an unfavourable response; all had viral load >400 copies/ml at 6 months. The NVP regimen proved to be inferior to the EFV regimen, the difference being significant ($P=0.008$).

At 1 month, plasma NVP and EFV values were available for 45 (favourable responders - 33) and 49 (favourable responders - 45) patients, respectively; at 6 months these were available for 33 (favourable responders - 26) and 48 patients (favourable responders - 44), respectively (Table II). The mean NVP and EFV levels did not significantly differ between favourable and unfavourable responders either at 1 or at 6 months (Table II). The difference in mean NVP concentrations between 1 (with RMP) and 6 months (without RMP) was significant (3.6 vs. 4.9 $\mu\text{g/ml}$, $P=0.003$); the corresponding difference in EFV concentrations (2.3 vs. 2.1 $\mu\text{g/ml}$) was not significant. Combining both regimens, no significant difference in the proportion of patients with sub-therapeutic NNRTI levels was observed between unfavourable and favourable responders either at 1 (56 versus 39%) or 6 (18 versus 28%) months. The number of patients with sub-therapeutic NNRTI concentrations at 1 month was higher than that at 6 months (42 vs. 28%); this difference was significant ($P=0.046$).

Genotyping of the *CYP2B6* 516G>T polymorphism was undertaken in 95 patients. Of these, the number of GG, GT and TT genotypes was 32, 45 and 18,

Table I. Characteristics of the patients included in the study

| | Nevirapine (NVP) | | Efavirenz (EFV) | |
|-------------------------------------|--------------------------------|---------------------------------------|-------------------------------|------------------------------------|
| | Baseline N=52 | 6 Month N=45 | Baseline N=55 | 6 Month N=52 |
| Age (yr) | 38.0 ± 7.9 | | 34.8 ± 7.5 | |
| Males (N) | 42 | | 46 | |
| Type of TB | | | | |
| Pulmonary | 27 | | 31 | |
| Extra-pulmonary | 25 | | 24 | |
| Body mass index | 16.3 ± 2.3 | 18.9 ± 2.4** | 16.3 ± 2.7 | 19.0 ± 2.7** |
| Haemoglobin (g/dl) | 9.5 ± 1.8* | 12.8 ± 2.7** | 10.3 ± 2.1* | 13.2 ± 1.9** |
| CD4+ cell counts (cells/μl) | 88 ± 61 | 297 ± 162** | 92 ± 60 | 308 ± 162** |
| Viral load (copies/ml) [†] | 228000 (93,500 - 5,73, 500) | 77600 [#] (7915 - 230000) | 322000 (78,100 - 7,50,000) | 3585 [#] (1198 - 7713) |

Values are mean ± SD
[†]Median (IQR); [#]denotes median (IQR) calculated for those with viral load >400 copies/ml - 10 in the NVP arm and 5 in the EFV arm;
*P=0.041 (NVP vs EFV at baseline); **P<0.001 compared to respective baseline

respectively; the genotype distribution followed Hardy-Weinberg equilibrium. Patients with the TT genotype had significantly higher plasma EFV concentrations compared to GG and GT genotypes ($P<0.001$). But differences in NVP concentrations among the genotypes did not attain statistical significance (Table II). Among 18 unfavourable responders for whom *CYP2B6* genotyping was done, 12 and 6 belonged to GG/GT and TT genotypes, respectively. Although a higher proportion of unfavourable responders belonged to the GG/GT than TT genotype, this difference was not significant.

Genotyping of the *CYP2B6* 983 T>C polymorphism undertaken in 95 patients showed that all belonged to the homozygous wild type and no mutations were observed.

The mean inter-patient variability of EFV and NVP was 117 and 59 per cent, respectively; the corresponding values for intra-patient variation were 34 and 32 per cent, respectively.

Logistic regression analysis by backward elimination method was performed to examine the association of variables such as patient's age, baseline body weight, baseline CD4 cell counts, *CYP2B6* genotype and plasma drug levels at 1 and 6 months with an unfavourable outcome. *CYP2B6* 516 G>T polymorphism was found to be significantly associated with outcomes in patients receiving EFV-based regimen; patients belonging to GG/GT genotype

Table II. Plasma nevirapine and efavirenz (NVP & EFV) levels in the different patient groups

| Variable | NVP (μg/ml) | EFV (μg/ml) |
|------------------------------------|---------------------|---------------------|
| Virologic outcome 1 month | | |
| Favourable responders | 3.8 ± 1.8 (n = 33) | 2.3 ± 2.5 (n = 45) |
| Unfavourable responders | 3.5 ± 1.6 (n = 12) | 2.5 ± 3.3 (n = 4) |
| 6 month | | |
| Favourable responders | 4.6 ± 2.0 (n = 26) | 1.9 ± 1.3 (n = 44) |
| Unfavourable responders | 6.5 ± 3.4 (n = 7) | 3.7 ± 5.0 (n = 4) |
| Rifampicin (RMP) co-administration | | |
| On RMP | 3.6 ± 1.7 (n = 49) | 2.3 ± 2.5 (n = 51) |
| Off RMP | 4.9 ± 2.5 (n = 35)* | 2.1 ± 1.9 (n = 49) |
| Genotype 1 month | | |
| GG | 3.3 ± 2.0 (n = 16) | 1.4 ± 0.9 (n = 14) |
| GT | 3.5 ± 1.6 (n = 20) | 1.5 ± 1.5 (n = 21) |
| TT | 4.1 ± 1.6 (n = 8) | 6.0 ± 3.0* (n = 10) |
| 6 month | | |
| GG | 4.4 ± 2.5 (n = 14) | 1.6 ± 0.8 (n = 16) |
| GT | 4.9 ± 2.6 (n = 15) | 1.7 ± 1.0 (n = 23) |
| TT | 6.1 ± 2.0 (n = 5) | 4.4 ± 3.4** (n = 8) |

Number of patients in each group given in parentheses
Values are mean ± SD
*P=0.003 vs. on RMP; **<0.001 vs. GG & GT genotypes

were more likely to have an unfavourable outcome ($P=0.022$).

At the end of ATT, 93 and 84 per cent of patients in the EFV and NVP arms, respectively had a favourable response. There were one treatment failure and three defaulters in the EFV arm and three deaths, three treatment failures and three defaulters in the NVP arm.

Discussion

The treatment of HIV infection requires prolonged use of antiretroviral drugs. Their effectiveness is often closely related to the achievement of adequate drug levels in blood^{5,14}. The utility of monitoring plasma NNRTIs to predict virological outcome remains a matter of debate. van Leth *et al*¹⁵ observed no relation between blood concentration and treatment response or occurrence of adverse events in adherent patients, thereby questioning the value of TDM. While several studies have reported an association between plasma NNRTIs and virological outcomes in HIV-infected patients without TB^{13,16,17}, a few studies in HIV-infected TB patients have yielded conflicting results. The N2R study from Thailand suggested that low drug exposure was an important predictive factor for treatment failure¹⁸. This prospective randomized trial in HIV-infected TB patients showed a significant correlation between treatment failure and reduced trough NNRTI levels. It was assumed that the higher rate of treatment failure in the NVP group during concomitant ATT with RMP was a result of sub-optimal trough levels compared to patients receiving EFV. Conversely, a lack of association between sub-therapeutic plasma NNRTI concentrations and virological failure in HIV-infected TB patients receiving ART and ATT has been reported by Sathia *et al*¹⁹. They observed favourable virological and immunological responses, high rates of successful TB therapy completion and microbiological cure. In both the studies, patients received a twice-daily NVP regimen.

The present study describes the association between plasma NVP and EFV and virological outcome in HIV/TB patients treated with a once-daily combination of antiretroviral drugs along with ATT. A higher proportion of patients had sub-therapeutic NNRTI concentrations at one month (during RMP co-administration) than at six months (without RMP). It is well established that RMP lowers plasma concentrations of NNRTIs, especially nevirapine, to sub-optimal levels. However, the key question is whether NVP or EFV (in the

standard dose) when administered along with RMP results in an increased risk of virological failure. Our trial showed that once-daily NVP regimen was inferior to EFV regimen with a higher rate of virologic failures and deaths, and that EFV was the drug of choice in HIV-infected patients with TB receiving rifampicin.⁶ In this pharmacokinetic sub-study, proportion of patients with sub-therapeutic drug concentrations (both NVP and EFV) did not differ between those with favourable and unfavourable responses. Thus, the clinical significance of NVP-RMP pharmacokinetic interaction resulting in reduced NVP concentrations during concomitant RMP treatment, and the cut-off values currently accepted, remain unclear. Several studies have reported on the lower efficacy of NVP-containing regimen compared to the EFV-containing regimen among HIV-infected TB patients^{6,20,21}. However, the role of sub-therapeutic plasma NVP during RMP co-administration in making the NVP regimen less effective remains a matter of conjecture. Further, pharmacokinetic studies capture blood levels only at certain time points and may not reflect the complete picture. Virologic failure in HIV-infected patients may be driven by several factors, and plasma NNRTI concentrations (using currently recommended definitions of therapeutic ranges) may not be the only determinant of treatment outcomes.

There were two patients for whom EFV was prematurely withdrawn (due to psychiatric manifestations) and two had NVP withdrawn (due to Stevens Johnson syndrome). Blood levels at one month were available for one patient each in the NVP and EFV arms. While EFV concentration was above the therapeutic range (5.12 µg/ml), NVP concentration was below the therapeutic range (2.6 µg/ml). Because of the very small number of patients with adverse effects in this study, plasma drug concentrations could not be associated with occurrence of the adverse reactions.

The study further demonstrated that individuals with a TT genotype of the *CYP2B6* 516G>T polymorphism had elevated trough levels of EFV with and without concomitant RMP treatment, a finding similar to that reported by Kwara *et al*²². Co-administration of RMP did not alter the impact of this polymorphism on the plasma concentration of EFV²³.

Logistic regression analysis showed that *CYP2B6* 516 G>T genotypes were significantly associated with virologic outcomes, among patients receiving EFV. Polymorphisms in this gene are also probably responsible for the wide inter-patient variability

observed in EFV blood levels. A higher proportion of patients belonging to GG/GT genotype among unfavourable responders and the association on multivariable regression suggested that patients with GG/GT genotypes were at a relatively higher risk of developing virological failure. These findings are similar to a study conducted in Thai HIV/TB co-infected patients receiving NVP or EFV; the authors observed that a higher proportion of TT genotypes achieved virologic suppression compared to GG and GT genotypes, though not statistically significant²⁴.

Genotyping of *CYP2B6* 983 T>C polymorphism in these patients showed that all patients were homozygous wild type (TT genotype). Wyen *et al*¹² had reported that the minor allele frequencies of this polymorphism were 0 and 0.07 in Caucasians and Blacks, respectively and that this polymorphism had a significant impact on plasma NVP and EFV. However, in the absence of individuals belonging to TC or CC genotypes, it is unlikely that *CYP2B6* 983 T>C polymorphism would play a role in influencing plasma NNRTI concentrations.

Our findings need to be interpreted in the context of the study design, which was to test once-daily antiretroviral regimens; the general practice is to treat with twice-daily regimens. Trough concentrations are known to be affected by the rhythm of administration; hence the findings may not be extrapolatable to situations where twice daily regimens are used. Further, we did not measure plasma levels during the first two weeks when NVP was given in a lead-in dose of 200 mg once-daily - with RMP having already induced the liver enzymes; it is possible that NVP levels were extremely low during this time, predisposing to virologic failure. Since this sub-study was nested within a randomized controlled clinical trial, a separate sample size and power calculation was not done. Other limitation of this study was the small number of patients with unfavourable outcomes and those belonging to the TT genotypes, diagnosis of TB pleuritis not based on microbiological, histopathological, or molecular evidence and risk due to interactions of NVP and RMP. The strengths of the study were that it was nested in a randomized clinical trial with fully supervised treatment, regular follow up and full ascertainment and documentation of outcomes.

This prospective study among patients receiving both ATT and ART showed no significant differences in plasma NNRTI concentrations between patients with

favourable and unfavourable outcomes. Polymorphisms in the *CYP2B6* gene, however, appeared to be correlated with virologic outcomes among patients on EFV. Further studies should attempt to determine the plasma levels of NNRTIs below which a poor response to treatment can be reliably predicted. Till then, therapeutic drug monitoring may not be a useful or cost-effective strategy for patients on ART.

Acknowledgment

The authors thank the nursing staff for blood collections, laboratory staff for investigations and Ms. Vennila for technical assistance in the HPLC laboratory, and also acknowledge Drs P.A. Menon, C. Padmapriyadarsini, G. Narendran, Sheikh Iliayas, S. Ramesh Kumar, P.K. Bhavani, Sriram Selvaraju and N. Pooranagangadevi for their assistance and support.

References

1. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, *et al*. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003; 163 : 1009-21.
2. Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the *in vitro* biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metab Dispos* 1999; 27 : 1488-95.
3. Kwara A, Ramachandran G, Swaminathan S. Dose adjustment of the non-nucleoside reverse transcriptase inhibitors during concurrent rifampicin-containing tuberculosis therapy: one size does not fit all. *Exp Opin Drug Metab Toxicol* 2010; 6 : 55-68.
4. Mallon P, Ray J, Cooper D. Effect of therapeutic drug monitoring on outcome in antiretroviral experienced HIV-infected patients. *J Clin Virol* 2003; 26 : 223-7.
5. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1 infected patients. *AIDS* 2001; 15 : 71-5.
6. Swaminathan S, Padmapriyadarsini C, Venkatesan P, Narendran G, Ramesh Kumar S, Sheik Iliayas, *et al*. Efficacy and safety of once daily nevirapine or efavirenz based antiretroviral therapy in HIV associated tuberculosis: A randomized clinical trial. *Clin Infect Dis* 2011; 53 : 716-24.
7. Petroff SA. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. *J Exp Med* 1915; 21 : 38-42.
8. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, *et al*. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969; 41 : 21-43.
9. Ramachandran G, Hemanth Kumar AK, Kumaraswami V, Swaminathan S. Simple liquid chromatography method for simultaneous determination of zidovudine and NVP in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006; 843 : 339-43.

10. Ramachandran G, Hemanth Kumar AK, Swaminathan S, Venkatesan, P, Kumaraswami V, Greenblatt DJ. Simple and rapid liquid chromatography method for determination of EFV in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006; 835 : 131-5.
11. Ariyoshi N, Miyazaki M, Toide K, Sawamura YI, Kamataki T. A single nucleotide polymorphism of *CYP2B6* found in Japanese enhances catalytic activity by autoactivation. *Biochem Biophys Res Comm* 2001; 281 : 1256-60.
12. Wyen C, Hendra H, Vogel M, Hoffmann C, Knechten H, Brockmeyer NH, *et al.* Impact of *CYP2B6* 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *J Antimicrob Chemother* 2008; 61 : 914-8.
13. Kappelhof BS, Crommentuyn KML, de Maat MMR, Mulder JW, Huitema ADR, Beijnen JH. Practical guidelines to interpret plasma concentrations of antiretroviral drugs. *Clin Pharmacokinet* 2004; 43 : 845-55.
14. Veldkamp AI, Weverling GJ, Lange JM, Montaner JSG, Reiss P, Cooper DA, *et al.* High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. *AIDS* 2001; 15 : 1089-95.
15. van Leth F, Kappelhoff BS, Johnson D, Losso MH, Boron-Kaczmarek A, Saag MS, *et al.* Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. *AIDS Res Hum Retroviruses* 2006; 22 : 232-9.
16. Havlir D, Cheeseman SH, McLaughlin M, Murphy R, Erice A, Spector SA, *et al.* High dose nevirapine: safety, pharmacokinetics, and antiviral effect in patients with human immunodeficiency virus infection. *J Infect Dis* 1995; 171 : 537-45.
17. Montaner J, Reiss P, Cooper D, Vella S, Harris M, Conway B, *et al.* A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS trial. Italy, The Netherlands, Canada and Australia study. *JAMA* 1998; 279 : 930-7.
18. Manosuthi W, Sungkanuparph S, Tantanathip P, Lueangniyomkul A, Mankatitham W, Prasithsirskul W, *et al.* A randomized trial comparing plasma drug concentrations and efficacies between 2 non-nucleoside reverse-transcriptase inhibitor-based regimens in HIV-infected patients receiving rifampicin: the N2R Study. *Clin Infect Dis* 2009; 48 : 1752-9.
19. Sathia L, Obiorah I, Taylor G, Kon O, O'Donoghue M, Gibbins S, *et al.* Concomitant use of non-nucleoside analogue reverse transcriptase inhibitors and rifampicin in TB/HIV type 1 co-infected patients. *AIDS Res Hum Retroviruses* 2008; 24 : 897-901.
20. Boule A, Van Cutsem G, Cohen K, Hilderbrand K, Mathee S, Abraham M, *et al.* outcomes of nevirapine and efavirenz based antiretroviral therapy when co-administered with rifampicin based anti-tubercular therapy. *JAMA* 2008; 300 : 530-9.
21. Manosuthi W, Manikatitham W, Lueangniyomkul A, Chimsuntorn S, Sungkanuparph S. Standard dose efavirenz vs. standard dose nevirapine in antiretroviral regimens among HIV-1 and tuberculosis co-infected patients who received rifampicin. *HIV Med* 2008; 9 : 294-9.
22. Kwara A, Lartey M, Sagoe KW, Xexemeku F, Kenu E, Oliver-Commy J, *et al.* Pharmacokinetics of efavirenz when co-administered with rifampin in TB/HIV co-infected patients: pharmacogenetic effect of *CYP2B6* variation. *J Clin Pharmacol* 2008; 48 : 1032-40.
23. Ramachandran G, Hemanth Kumar AK, Rajasekaran S, Kumar P, Ramesh K, Anitha S, *et al.* *CYP2B6* G516T polymorphism but not rifampin co-administration influences steady state pharmacokinetics of efavirenz in human immunodeficiency virus-infected patients in south India. *Antimicrob Agents Chemother* 2009; 53 : 863-8.
24. Uttayamkul S, Likonansakul S, Manosuthi W, Wichukchinda N, Kalambaheti T, Nakayama EE, *et al.* Effects of *CYP2B6* G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected adults. *AIDS Res Ther* 2010; 7 : 8-17.

Reprint requests: Dr Soumya Swaminathan, Director, National Institute for Research in Tuberculosis (Formerly Tuberculosis Research Centre), Mayor V.R. Ramanathan Road, Chetput, Chennai 600 031, India
e-mail: doctorsoumya@yahoo.com