

APPLIED RESEARCH



Studies completed:

Pharmacokinetics of generic fixed-dose combinations of nevirapine, lamivudine and stavudine in HIV-1 infected adults in India

Background:

Generic Fixed Dose Combinations (FDC) of regimens containing two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and one Non-NRTI (NNRTI) are widely regarded as crucial in the scaling up of AIDS treatment in developing countries.

Two triple drug combinations of nevirapine (NVP) and lamivudine (3TC) with either stavudine (d4T) or zidovudine (AZT) as the third drug are available as FDCs in the developing world. Since very limited data is available on the blood levels or pharmacokinetics of these drugs in Indian patients, a study was undertaken with the following aims:

Aims:

1. To obtain information on the pharmacokinetic profiles of NVP, 3TC and d4T in HIV-infected patients on treatment with FDCs in India.
2. To study the influence of immunological status, sex and body mass index (BMI) on the pharmacokinetics of these drugs.

Methods:

HIV – infected adults attending the outpatient clinic of the centre during October 2004 to September 2005 and undergoing treatment with generic FDC of antiretroviral drugs (NVP 200 mg /3TC 150mg/ d4T 30/40 mg or AZT 300 mg bi-daily) for a minimum period of two weeks, participated in the study. The study was conducted at the Government Hospital of Thoracic Medicine, Tambaram, Chennai. On the day of the study, serial blood samples were collected at different time points after administration of the FDC pill. Plasma NVP, 3TC and d4T were estimated by validated HPLC methods. Based on the plasma concentrations, certain pharmacokinetic variables were calculated using WinNonlin software.

Results:

Twenty nine HIV-infected patients took part in the study. Their baseline characteristics are given in Table 8. The steady state pharmacokinetics of NVP, 3TC and d4T are shown in Table 9. Peak and trough concentrations and exposure of NVP were higher in Indians than American and European populations. But it was similar to that reported in Malawians. The pharmacokinetic profile of 3TC and d4T in Indians was almost similar to that reported by others. The degree of immune suppression, sex and BMI did not have any impact on the pharmacokinetics of NVP, 3TC and d4T. Although, a significant difference in peak concentration of d4T between patients with CD4 cell counts = 200 cells/mm³ (0.33µg/ml) and > 200 cells/mm³ (0.53µg/ml), was observed (P < 0.05), these values are within the therapeutic range of the drug.



Table 8: Baseline characteristics of study participants

Characteristics	Value
Sex (No)	
Males	19
Females	10
Age (Years)	
Mean	36
Range	26-50
Body Weight (kg)	
Mean	52
Range	35-91
Height (cm)	
Mean	159
Range	140-173
BMI	
Mean	20.3
Range	14.5-33.0
Duration of ARTI (months)	
Mean	4.4
Range	1-17
CD4 counts (cells/mm ³)	
Mean	218
Range	25-684
≥ 200 cells/mm ³ (No.)	16
<200 cells/mm ³ (No.)	13

Conclusion:

Adequate plasma concentrations of NVP, 3TC and d4T that are not influenced by the stage of immune suppression, gender and BMI observed in this study, are quite encouraging. Hence, if patients take regular treatment, chances of failure due to inadequate drug levels are low. The relatively higher steady state plasma NVP concentrations observed in Indian patients indicate the need to explore pharmacogenetic differences that could impact drug levels in different populations.

Table 9: Steady state pharmacokinetics of nevirapine, lamivudine and stavudine

Mean ± SD					
Drug	C _{max} (µg/ml)	C _{min} (µg/ml)	T _{max} (hours)	AUC ₀₋₁₂ (µg/ml-hours)	t _{1/2} (hours)
Nevirapine n = 26	8.50 ± 2.44	5.05 ± 2.04	1.38 ± 0.83	80.69 ± 26.85	29.82 ± 11.66
Lamivudine n = 27	2.39 ± 0.61	0.27 ± 0.13	1.5 ± 0.90	11.63 ± 2.99	4.48 ± 1.84
Stavudine n = 14	0.42 ± 0.18	0.025 ± 0.003	0.89 ± 0.40	1.45 ± 0.42	3.23 ± 1.92

C_{max} - peak concentration; C_{min} - trough concentration; T_{max} - Time to attain C_{max}; AUC - Area under the plasma concentration vs. time curve.



Studies in progress:

Screening for CYP2B6 (G516T) polymorphisms in HIV-infected patients in India

Background:

The use of combinations of antiretroviral drugs to provide potent ART, has dramatically improved the morbidity and mortality due to HIV infection and AIDS. Investigation of host genetic factors that impact both the efficacy and toxicity of ART, may aid in selecting the best regimen for individual patients. The non-nucleoside reverse transcriptase inhibitors, efavirenz (EFV) and NVP are used as a first-line treatment of HIV-infected patients in India along with nucleoside reverse transcriptase inhibitors.

Plasma concentrations of EFV are known for a high degree of inter-patient variability. Similar variability is observed with NVP. These drugs are metabolized by the cytochrome P450 2B6 (CYP2B6), an isoenzyme characterized by wide inter-individual variability in expression and activity in human livers *in vitro*. A CYP2B6 allelic variant in exon 4 (G516T, Gln172His), has been reported to influence EFV and NVP pharmacokinetics in Japanese, European-Americans and African-Americans. Identification of population differences in the frequency of polymorphism in the gene that encodes CYP2B6 is a matter of concern, since genetic differences among populations could lead to differences in antiretroviral drug concentrations, tolerability and outcome.

Information on the frequency of CYP2B6 polymorphisms in an Indian population and its association with EFV and NVP blood levels is lacking. It has therefore been planned to carry out pharmacogenetic studies with the following aims:

Aims:

1. To study the frequency distribution of CYP2B6 polymorphisms by genotyping for substitution at position 516 in exon 4 (G>T) in HIV-infected patients in south India.
2. To assess the influence of CYP2B6 polymorphisms on plasma concentrations of EFV and NVP.

Methods:

HIV-infected subjects receiving EFV (600mg once daily) or NVP (200mg twice daily) along with two nucleoside analogues for a minimum period of 15 days at the Government Hospital of Thoracic Medicine, Tambaram will be included. Plasma samples will be collected at 12 hours for EFV and 2 hours for NVP after drug administration. The drug concentrations will be analysed by High Performance Liquid Chromatography (HPLC). Genetic characterization of the CYP2B6 gene at position 516 will be performed by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) analysis using genomic Deoxyribonucleic Acid (DNA) extracted from whole blood. The primers, forward (5' CTTGACCTGCTGCTTCTCC 3') and reverse (5' TCCCTCTCCGTCTCCCTG 3') will be used to amplify a 204 base pair product. The PCR product will be digested with



BsrI at 65°C overnight. Based on the number and size of the fragments, the subjects will be classified as GG, GT or TT genotype. Genotypes will be identified by the pattern of bands observed as follows:

GG	2 fragments, 152, 52 bp
GT	3 fragments, 204, 152, 52 bp
TT	1 fragment, 204 bp

The different genotypes will be correlated with 12-hour concentration in the case of EFV and 2-hour concentration in the case of NVP.

Fifty patients each receiving NVP and EFV will be included in to the study.

So far, genomic DNA has been extracted from 25 patients on NVP regimen and 15 patients on EFV regimen. PCR-RFLP analyses of these samples are in progress.

Pharmacokinetics of efavirenz during anti-TB treatment with rifampicin-containing regimens

Background:

EFV, a non-nucleoside reverse transcriptase inhibitor, has been recommended as a first line option in the ART and the preferential choice in TB and HIV co-infected patients. The available pharmacokinetic data provide evidence for only a 13-25 per cent reduction in EFV levels, when co-administered with RMP. This is lower than nevirapine (40 per cent) and protease inhibitors (80-95 per cent).

Despite few studies demonstrating favorable clinical outcomes in HIV-TB patients receiving EFV and RMP concomitantly, serious concerns have been raised regarding the adequacy of the conventional dose of EFV (600mg once daily) when co-administered with RMP—particularly in patients with higher body weight. At present there is no information available on EFV pharmacokinetics and the effect of RMP on EFV metabolism in Indian subjects. Therefore a study was planned with the following aims:

Aims:

1. To compare peak, trough levels and exposure of EFV in HIV and TB co-infected patients during and after completion of anti-TB treatment with RMP-containing regimens.
2. To correlate blood levels of EFV with body weight of patients.

Methods:

The study is being conducted in collaboration with the Government Hospital of Thoracic Medicine, Tambaram. The study participants will comprise of adult HIV-TB patients, undergoing treatment regularly with EFV and RMP containing antiretroviral and anti-TB regimens respectively, for a minimum of 1 week and willing to give informed consent. It is proposed to conduct the study amongst 30 HIV-TB patients.

All patients will be investigated on 2 occasions – once while receiving EFV and RMP-containing antiretroviral and anti-TB regimens, and next a month after completion of anti-TB treatment. During both the occasions, serial blood samples



will be collected at different time points after oral administration of EFV (600 mg). Estimation of plasma EFV will be carried out by HPLC. Based on the plasma concentrations of EFV and RMP at different time points, certain pharmacokinetic variables will be calculated.

The pharmacokinetic variables of EFV obtained during the anti-TB treatment (occasion I), will be compared with those obtained after completion of anti-TB treatment (occasion II). The per cent change in EFV pharmacokinetics in the presence of RMP, will be calculated. Correlation between pharmacokinetic variables of EFV and body weight will be calculated. So far, fifteen patients have completed the first occasion of the study.

Studies completed:

Candidal antibody response in serum and saliva of HIV-infected patients with and without oral Candidiasis

Background:

HIV-infected individuals are predisposed to recurrent oral candidiasis. A study was carried out to investigate the role of humoral immunity by comparing the concentrations of IgA and IgG antibodies to *Candida albicans* in whole saliva and serum samples from HIV-infected patients, with and without oral thrush.

Methods:

The study target comprised of 14 HIV seropositive patients with oral thrush (HIV+ oral thrush), 16 HIV seropositive patients without thrush (HIV+) and 13 healthy controls (HIV-). The anti candidal IgG titer was measured in saliva and serum using commercial ELISA kits.

Results:

In all the three groups, the IgG levels were higher in serum while IgA levels were higher in saliva. The HIV+ oral thrush group had significantly higher levels of candida specific IgA (45.3 ± 4.1 Vs 31.2 ± 2.4) and IgG (31.5 ± 5.7 Vs 14 ± 1.4) in the saliva compared to normals ($p < 0.05$). There was no correlation between CD4 counts and IgG or IgA levels.

Conclusion:

These results suggest that HIV seropositive individuals are able to mount a good local antibody response. A defect in the mucosal humoral immune response in the oral cavity in HIV seropositive individuals does not appear to be responsible for the increased prevalence of oral candidiasis. High levels of candida specific IgA and IgG in saliva do not protect the patients from developing oral thrush. Further studies on cellular immunity have to be done to relate defective local immune response and the increased prevalence of oral candidiasis in HIV seropositive individuals.

Seroprevalance of Herpes simplex Virus –1 & 2 antibodies in HIV Positive and HIV negative individuals in south India

Background:

The Herpes Simplex Virus (HSV) is quite common through out the world. It is generally associated with oral (HSV-1) or genital ulcers (HSV-2) and is transmitted heterosexually. A large proportion of individuals with serologic infection with HSV



are asymptomatic. HSV-2 is a significant public health hazard because of its potential role as a cofactor in HIV transmission. A study was carried out to compare the sero prevalence of HSV-1 and HSV-2 infection in HIV sero positive and sero negative individuals in south India.

Materials and Methods:

A cohort of 70 HIV-infected and uninfected individuals, were included in this prospective study (35 HIV-infected and 35 HIV uninfected). All serum samples were randomized and assessed for HSV-1 and HSV-2 IgG and IgM antibodies with HSV type specific Enzyme Linked Immunosorbant Assay (ELISA).

Results:

In the HIV positive group, out of 35 individuals screened for HSV-1 antibodies, 34.3 per cent were positive for IgM and 80 per cent were positive for IgG antibodies. When screened for HSV-2 antibodies, the frequencies for IgM and IgG were 28.6 per cent and 60 per cent respectively. In the HIV negative group, out of 35 individuals screened for HSV-1 antibodies, the occurrence of IgM and IgG antibodies were 34.3 per cent and 71.4 per cent respectively, while the antibodies for HSV-2, showed prevalence rate of 17.1 per cent for both IgM and IgG. Using the rate chi-squared test, it was observed that there is a relationship between the presence of HSV-2 antibody and the HIV status. There is a significant difference between HIV positive and HIV negative individuals in prevalence of HSV-2 IgG antibodies ($P < 0.05$).

Conclusion:

A higher rate of HSV-2 IgG antibody was found in HIV-infected individuals as compared to HIV negative individuals. This could have increased the risk for HIV acquisition.

Patients with TB have cross reacting antibodies against HIV-1

Some individuals produce antibodies that react with HIV-1 proteins, but are not diagnostic for HIV infection. Western blots from patients with TB, were analyzed for the presence of cross-reacting antibodies to HIV antigens. A total of 153 TB serum samples were analyzed for the presence of reactive bands against HIV antigens. All 153 patients who were HIV negative, showed varied bands to the HIV-1 antigens on western blot and Line Immuno Assay (LIA). Band appearance to the pol gene products (p-66, p-55, p-51, p-31) of HIV-1 appeared most frequently. A few bands were produced against the HIV-1 env proteins (gp-120 and gp-41). Bands against the gag proteins were also seen (p-24 and p-17). Cross-reactivity was seen more commonly with LIA than with western blot. A cladistic protein parsimony approach and a distance based neighbor-joining approach, were used to evaluate and identify the cross reacting antigens in *M. tuberculosis*. A phylogenetic tree was constructed between HIV-1 subtype C antigen sequences and *M. tuberculosis* CDC1551 antigen sequences.

It appears that TB patients could exhibit false positive antibody reactions against HIV antigens and that some *M. tuberculosis* antigens may have a similar parental gene as HIV. Patients with active TB have HIV-1 cross-reacting antibodies on western blot. This may interfere with the HIV-1 diagnostic testing. Further research is required to analyze the relation between HIV and *M. tuberculosis* antigens *in vitro*. Results of these studies may provide an insight on why such non diagnostic bands are frequent in HIV testing.