ORIGINAL RESEARCH ARTICLE

Minimum effective plasma concentration of efavirenz in treatment-naïve Chinese HIV-infected patients

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Summary: To assess the relationship between mean efavirenz (EFV) plasma concentration and clinical effect during the first 48 weeks of combination antiretroviral therapy (cART), the baseline CD4 cell count was recorded and plasma EFV concentration and CD4 cell count were measured every 12 weeks. HIV-RNA viral load was determined in the 48th week of cART. In total, 42 subjects were recruited and grouped according to their mean concentration of EFV during the study period: groups A, B and C with EFV concentrations (mean) <2 mg/L (1.8 mg/L), 2–4 mg/L (2.9 mg/L) and >4 mg/L (5.5 mg/L), respectively. The CD4 cell counts in group C increased more quickly than in groups B and A, although this was not statistically significant (211 $\pm$ 176/μL versus 151 $\pm$ 145/μL and 172 $\pm$ 105/μL, respectively; $P = 0.799$). Groups B and C had higher rates of HIV viral load suppression than group A ($P = 0.017$). For treatment-naïve Chinese HIV-infected patients, EFV plasma concentrations above 2 mg/L appear to suppress HIV replication more effectively than concentrations below 2 mg/L.

Keywords: combination antiretroviral therapy, therapeutic drug monitoring, efavirenz, plasma concentration, HIV replication

INTRODUCTION

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor for the treatment of HIV-1-infected individuals. Its long half-life (40–55 hours) allows once-daily dosing and therefore brings the advantage of better treatment adherence and efficacy.[1] However, the emergence of EFV-resistant strains is likely to be facilitated by exposure to subtherapeutic drug levels, and treatment failure is more frequent in patients with levels lower than 1 mg/L compared with those with higher levels.[2] Other reports suggest that raising the lower level of the therapeutic range to 2 mg/L results in better treatment responses.[3] Because of pharmacokinetic differences among infected individuals, high values of interindividual variability in EFV plasma concentrations are observed (coefficient of variation [CV] $>$ 55%).[2–4] To date, many studies have focused on the therapeutic drug monitoring (TDM) of EFV, and some[2,5,6] have been based on a single sample for evaluating long-term therapy effects, and yet these studies overlooked the fact that considerable intrapatient variability in EFV concentrations may result in differences in plasma concentration over time.[5,7] Additionally, some studies[8–12] recruited both treatment-naïve and treatment-experienced subjects, potentially clouding any associations between plasma concentration and clinical effect.

Studies utilizing regular blood sampling to analyse the association between EFV concentrations and clinical effects are scarce. In the present study, we assessed mean EFV plasma concentrations by sampling 10–15 hours post dosing 12 weeks after therapy and collecting regular data from subjects during their first 48 weeks of follow-up. This allowed for analysis of the association between mean EFV concentrations over the whole 48 weeks and HIV surrogate markers. This study was carried out among treatment-naïve Chinese patients; with limited medical resources in China, strategies to prevent the emergence of EFV-resistant HIV are needed and our findings may help guide local treatment strategies.

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METHODS

Study design

The protocol received prior approval from the Shanghai Public Health Clinical Center Ethics Committee. From January 2008 to December 2009, patients were recruited and excluded according to the following criteria. Inclusion criteria were HIV antibody positive, naïve to ART, CD4 cell count below 350/μL, intention to start an EFV-based combined antiretroviral therapy (cART) regimen, age $\geq$18 years, and, in female patients, willingness to use birth control. Informed consent was obtained from all participants. Exclusion criteria included active opportunistic infection or AIDS-related cancer, chronic diarrhoea, malabsorption, active drug or alcohol abuse, less than four samples for EFV concentration measurement during the 48 weeks' follow-up and currently pregnant or breast-feeding women. Those patients who failed to take EFV more than twice monthly were regarded excluded from this study due to poor

adherence. Adherence was measured with a questionnaire administered by clinicians.

When patients were recruited to this research, general clinical characteristics of subjects were recorded such as age, body weight and body mass index (BMI). Hepatitis B and C status and baseline CD4 cell count were determined and subsequently blood was collected every 12 weeks for EFV plasma concentrations and CD4 cell counts. At the same time, clinicians recorded symptoms of EFV-related central nervous system (CNS) toxicity: dizziness, insomnia, impaired concentration, somnolence, abnormal dreams and hallucinations. HIV-RNA viral loads of all the subjects were performed in the 48th week after cART. Twelve weeks after initiating their regimens, plasma samples for EFV concentrations in all subjects were drawn during the morning, 10–15 hours post dosing. HIV-RNA and CD4 cell counts were quantified in the local clinical laboratory where the hepatitis serology was also performed.

Quantification of drug concentration was determined as described previously[13] Detection of EFV was performed on a triple quadrupole mass spectrometer. The mobile phase consisted of A (Water): B (Acrylonitrile) (50:50, v/v). Lower limit of EFV quantitation (LLOQ) is 50 ng/mL and mean EFV intra- and inter-day precision were less than 11% with accuracy between 85% and 115%.

Statistics

Results were shown as mean ± standard deviation (SD) or median and interquartile range (IQR) or percentage (%). Demographic factors such as age, BMI and weight were assessed by linear regression to search for potential relationships with the $\log_{10}$ EFV plasma concentration. Differences between groups of patients (absolute values or % change) were compared with analysis of one-way analysis of variance or Wilcoxon Rank sum test and Pearson χ^2. STATA (Statistics Data Analysis 7.0, TX, USA) was used for statistical analyses. Statistical significance was assumed at $P < 0.05$.

RESULTS

There were initially 48 eligible HIV-1-infected patients recruited; six subjects were subsequently excluded because of irregular follow-up or poor adherence. Among the 42 patients, 32 (76%) subjects received zidovudine (AZT) + lamivudine (3TC) + EFV as their regimen while 10 (24%) patients used stavudine (D4T) + 3TC + EFV. Every participant had four measurements during the whole follow-up and a total of 168 samples were collected.

The concentration of 168 samples ranged from 0.875 to 18.12 mg/L, and only one sample concentration was below 1 mg/L. The median EFV plasma concentration throughout the follow-up period was 3.3 mg/L (IQR 2.1–4.1 mg).

Patients' ages ranged from 23 to 59 years, with mean age of 41 ± 11 years, and their average body weight was 64.2 ± 9.0 kg while the mean BMI was 21.7 ± 2.7 kg/m^2. There was no relationship observed between mean EFV concentration and patient age or body weight. There were eight patients co-infected with hepatitis B or C and among the 42 subjects, six (14%) were women; EFV levels were not affected by gender or hepatitis status. A relationship between EFV concentration and CNS toxicity was not observed (see Table 1).

Table 1 Association between patient demographics, clinical data and EFV concentration

Variable	Efavirenz concentration (mg/L)		*P* value
	Median	IQR	
Efavirenz concentration (mg/L)			
Age*	–	–	0.183
Weight*	–	–	0.704
BMI*	–	–	0.907
Sex	–	–	0.733
Male ($n = 36$)	3521	2156–4036	
Female ($n = 6$)	3008	1878–4189	
Co-infection with HBV/HCV	–	–	0.620
Yes ($n = 8$)	3713	2138–5174	
No ($n = 34$)	3386	2115–4063	
CNS toxicity	–	–	0.736
Yes ($n = 9$)	3332	2326–4450	
No ($n = 33$)	3480	1998–4086	

BMI = body mass index; CNS = central nervous system; EFV = efavirenz; IQR = interquartile range

*Relationship between continuous variables and $\log_{10}$ EFV plasma concentration was assessed by linear regression. All other variables were assessed by Wilcoxon rank sum test

Subjects were grouped according to mean EFV concentrations during the study period: groups A, B and C with EFV concentrations <2, 2–4 and >4 mg/L, respectively. The virological and immunological outcomes among these groups after 48 weeks of cART are summarized in Table 2.

In univariate analysis, groups B and C were more likely to have a viral load <50 copies/mL than group A, but no statistically significant differences were observed between groups B and C.

The baseline CD4 level was not statistically different among these groups at baseline or during follow-up; CD4 increase after 48 weeks of cART in the different groups is depicted in Figure 1.

DISCUSSION

In this study, EFV was taken at bedtime to minimize its adverse effects; however, blood samples were taken during the morning in the outpatient clinic. Marzolini *et al.*[2] demonstrated that, given the long half-life of EFV, sampling at 8–20 hours post dosing only influences the total variance of EFV concentrations slightly (3%) and the detection of EFV plasma concentration based on samples taken between eight and 20 hours post dosing has been reported in other studies.[7,14,15] In the present

Table 2 **Relationship between mean EFV concentration and surrogate markers after 48 weeks of cART**

Analysis per patient		Efavirenz concentration			*P* value
		<2 mg/L $n = 8$	2–4 mg/L $n = 22$	>4 mg/L $n = 12$	
Baseline CD4 cell count ($\times 10^6$/L)	Mean ± SD	204 ± 102	205 ± 105	145 ± 128	0.418
Viral load <50 copies/mL after 48 weeks cART	Yes (%)	2 (25)	16 (73)	10 (83)	0.017
Count increase during the whole follow-up	Mean ± SD	172 ± 105	151 ± 145	211 ± 176	0.799

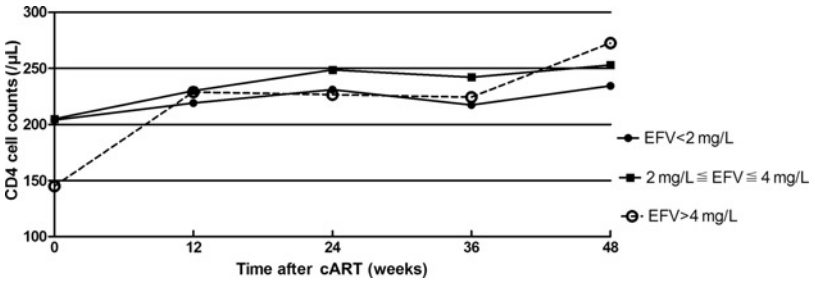

Figure 1 **Increase of CD4 cell count of three groups with different EFV concentration levels after cART. EFV= efavirenz; cART= combination antiretroviral therapy**

study, we regard EFV levels measured by this method as trough concentrations. EFV is extensively metabolized to inactive hydroxylated metabolites by the cytochrome P450 enzymatic system,[16,17] and changes in the hepatic drug metabolism of HIV patients co-infected with hepatitis B and C can be affected.[15] However, as in previous reports,[15] we did not find significant differences in EFV levels between patients with and without co-infection, perhaps because hepatic function was well preserved in our subjects.[18]

As for the relationship between age and EFV metabolism, there are no definite conclusions. Some researchers[19,20] demonstrated that no association between age and EFV concentration level was found. However, Sotaniemi *et al.*[21] suggested that ageing could impact on drug metabolism, showing approximately 30% decline in CYP450 content after 70 years compared with subjects aged 20 to 29 years. In our patients, the age ranged from 23 to 59 years and an association between age and EFV concentration was not found; further research with larger numbers of older patients is needed.

Burger *et al.*[19] reported that women were more liable to have EFV-related toxicity because of higher EFV concentrations than men of the same ethnicity. However, in some other studies, there was no correlation with gender and EFV pharmacokinetics.[4,22] In Caucasian, African or Hispanic Americans, marked gender differences in CYP2B6 expression and its activity have been observed.[23] In comparison, the frequencies of mutations in Han Chinese women were higher than those in men,[24] but there were no significantly statistical differences. Our research did not find an impact of gender on EFV level. This phenomenon might partly be explained by the different CYP2B6 variants' distribution or expression between men and women in different races.[23]

Many previous studies[25–27] reported that high EFV levels increase the risk of adverse effects such as CNS toxicity. However, this effect was not seen in our small study. Clifford *et al.*[27] reported that most adverse effects related to EFV happened during the first two to four weeks of treatment; as we only collected adverse effect data after 12 weeks cART this might explain the disparity between our results and other studies.[2,28] Moreover, our data on CNS side-effects were based on written patient records and not on specific measurement of such features; hence, our ability to find a relationship might have been reduced.

As for the 'therapeutic range' of EFV concentration, Marzolini *et al.*[2] suggested that the interval 1–4 mg/L would be optimal for patients. Ståhle *et al.*[3] demonstrated that a 70% antiretroviral response rate could be reached at the lower limit of 1 mg/L while more than 80% response rate at the lower bound of 2 mg/L. Based on previous research,[29] Chinese patients would have higher EFV concentration because of the difference of CYP2B6 polymorphisms between Chinese and Caucasian individuals. Among the 168 blood samples, there was only one sample with an EFV concentration below 1 mg/L; thus, our research took 2 mg/L as the lower limit of the EFV therapeutic range. We found that EFV levels above 2 mg/L were more likely to achieve complete HIV suppression than EFV levels below 2 mg/L. However, there were no significant differences of HIV suppression between EFV levels above 4 mg/L and between 2 and 4 mg/L. Moreover, there was no association between adverse effects related to EFV and its concentration, and so EFV dose reductions in patients with concentrations greater than 4 mg/L would not necessarily ameliorate toxicity and might reduce the beneficial effects of EFV on HIV suppression.

Although baseline CD4 cell counts among the groups with different mean EFV concentrations were similar, during the 48-week follow-up the mean CD4 cell count increases of the three groups were not statistically different. Catalfamo *et al.*[30] reported that cART could increase the number of CD4 cells by suppressing HIV replication, but many other factors could also impact the increase of CD4 cells during cART.[31,32] Hence, the exact relationship between the increase of CD4 cell counts and HIV suppression remains unclear.[33] This might explain why the increase in CD4 cell counts among patients with different EFV levels was not significantly different after 48 weeks of follow-up.

The sample size of this study was relatively small, and only six female subjects were enrolled. This should be considered when interpreting these results and larger cohorts are needed to strengthen our findings.

In conclusion, patients with EFV levels above 2 mg/L may be more likely to achieve complete HIV suppression than those with EFV levels below 2 mg/L. During the first year of EFV-containing cART, blood sampling to determine EFV levels below 2 mg/L in Chinese patients may identify those patients less likely to achieve HIV suppression. In the absence of regular virological monitoring this may offer an alternative strategy to complement adherence counselling and support.

ACKNOWLEDGEMENT

Financial support was provided by the AIDS prevention & treatment research programme of the China Ministry of Health (No. WA-2007-04) and the Key Technological and Scientific Programme of the National 11th Five-Years Project (The research of anti-TB treatment strategy among AIDS patients with tuberculosis co-infection, No. 2008ZX10001-008.) The authors are grateful

to Qi Tangkai, Jiang Xueyan, Wang Zhenyan, Li Li, Sun Fuyan and Pan Chengen for clinical support and Jia Xiaofang, Peng Xia, Ma Jianxin and Wang Xinlei for laboratory technical support. J Sun and J Chen contributed equally to this paper.

REFERENCES

1 Adkins JC, Noble S. Efavirenz. *Drugs* 1998;**56**:1055–64
2 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
3 Stahle L, Moberg L, Svensson JO, Sonnerborg A. Efavirenz plasma concentrations in HIV-infected patients: inter and intraindividual variability and clinical effects. *Ther Drug Monit* 2004;**26**:267–70
4 Csajka C, Marzolini C, Fattinger K, *et al.* Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clin Pharmacol Ther* 2003;**73**:20–30
5 Nettles RE, Kieffer TL, Parsons T, *et al.* Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin Infect Dis* 2006;**42**:1189–96
6 Fabbiani M, Di Giambenedetto S, Bracciale L, *et al.* Pharmacokinetic variability of antiretroviral drugs and correlation with virological outcome: 2 years of experience in routine clinical practice. *J Antimicrob Chemother* 2009;**64**:109–17
7 Pereira SA, Branco T, Caixas U, *et al.* Intra-individual variability in efavirenz plasma concentrations supports therapeutic drug monitoring based on quarterly sampling in the first year of therapy. *Ther Drug Monit* 2008;**30**:60–6
8 Bossi P, Peytavin G, Ait-Mohand H, *et al.* GENOPHAR: a randomized study of plasma sdrug measurements in association with genotypic resistance testing and expert advice to optimize therapy in patients failing antiretroviral therapy. *HIV Med* 2004;**5**:352–9
9 Burger D, Hugen P, Reiss P, *et al.* ATHENA Cohort Study Group. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naive HIV-1-infected individuals. *AIDS* 2003;**17**:1157–65
10 Clevenbergh P, Garraffo R, Durant J, Dellamonica P. PharmAdapt: a randomized prospective study to evaluate the benefit of therapeutic monitoring of protease inhibitors: 12 week results. *AIDS* 2002;**16**:2311–5
11 Fletcher CV, Anderson PL, Kakuda TN, *et al.* Concentration-controlled compared with conventional antiretroviral therapy for HIV infection. *AIDS* 2002;**16**:551–60
12 Khoo SH, Lloyd J, Dalton M, *et al.* Pharmacologic optimization of protease inhibitors and nonnucleoside reverse transcriptase inhibitors (POPIN) – a randomized controlled trial of therapeutic drug monitoring and adherence support. *J Acquir Immune Defic Syndr* 2006;**41**:461–7
13 Pelerin H, Compain S, Duval X, Gimenez F, Benech H, Mabondzo A. Development of an assay method for the detection and quantification of protease and non-nucleoside reverse transcriptase inhibitors in plasma and in peripherical blood mononuclear cells by liquid chromatography coupled with ultraviolet or tandem mass spectrometry detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2005;**819**:47–57
14 Langmann P, Weissbrich B, Desch S, *et al.* Efavirenz plasma levels for the prediction of treatment failure in heavily pre-treated HIV-1 infected patients. *Eur J Med Res* 2002;**7**:309–14
15 Pereira SA, Caixas U, Branco T, *et al.* Efavirenz concentrations in HIV-infected patients with and without viral hepatitis. *Br J Clin Pharmacol* 2008;**66**:551–5
16 Mutlib AE, Chen H, Nemeth G, Gan LS, Christ DD. Liquid chromatography/mass spectrometry and high-field nuclear magnetic resonance characterization of novel mixed diconjugates of the non-nucleoside human immunodeficiency virus-1 reverse transcriptase inhibitor, efavirenz. *Drug Metab Dispos* 1999;**27**:1045–56
17 Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J Pharmacol Exp Ther* 2003;**306**:287–300
18 Barreiro P, Rodriguez-Novoa S, Labarga P, *et al.* Influence of liver fibrosis stage on plasma levels of antiretroviral drugs in HIV-infected patients with chronic hepatitis C. *J Infect Dis* 2007;**195**:973–9
19 Burger D, van der Heiden I, la Porte C, *et al.* Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of gender, race, and CYP2B6 polymorphism. *Br J Clin Pharmacol* 2006;**61**:148–54
20 Rodriguez-Novoa S, Barreiro P, Rendon A, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Influence of 516G>T polymorphisms at the gene encoding the CYP450–2B6 isoenzyme on EFV plasma concentrations in HIV-infected subjects. *Clin Infect Dis* 2005;**40**:1358–61
21 Sotaniemi EA, Arranto AJ, Pelkonen O, Pasanen M. Age and cytochrome P450-linked drug metabolism in humans: an analysis of 226 subjects with equal histopathologic conditions. *Clin Pharmacol Ther* 1997;**61**:331–9
22 Lamba V, Lamba J, Yasuda K, *et al.* Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J Pharmacol Exp Ther* 2003;**307**:906–22
23 Pfister M, Labbe L, Hammer SM, *et al.* Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: an Adult AIDS Clinical Trial Group Study 398. *Antimicrob Agents Chemother* 2003;**47**:130–7
24 Guan S, Huang M, Chan E, Chen X, Duan W, Zhou SF. Genetic polymorphisms of cytochrome P450 2B6 gene in Han Chinese. *Eur J Pharm Sci* 2006;**29**:14–21
25 Cespedes MS, Aberg JA. Neuropsychiatric complications of antiretroviral therapy. *Drug Saf* 2006;**29**:865–74
26 Haas DW, Ribaudo HJ, Kim RB, *et al.* Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group Study. *AIDS* 2004;**18**:2391–400
27 Clifford DB, Evans S, Yang Y, *et al.* Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. *Ann Intern Med* 2005;**143**:714–21
28 Gutiérrez F, Navarro A, Padilla S, *et al.* Prediction of neuropsychiatric adverse events associated with long term efavirenz therapy, using plasma drug level monitoring. *Clin Infect Dis* 2005;**41**:1648–53
29 Xu BY, Guo LP, Lee SS, *et al.* Genetic variability of CYP2B6 polymorphisms in four southern Chinese populations. *World J Gastroenterol* 2007;**13**:2100–3
30 Catalfamo M, Di Mascio M, Hu Z, *et al.* HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T cells. *Proc Natl Acad Sci USA* 2008;**105**:19851–6
31 Teixeira L, Valdez H, McCune JM, *et al.* Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS* 2001;**15**:1749–56
32 Backus LI, Phillips BR, Boothroyd DB, *et al.* Effects of hepatitis C virus coinfection on survival in veterans with HIV treated with highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2005;**39**:613–9
33 Henry WK, Tebas P, Lane HC. Explaining, predicting, and treating HIV-associated CD4 cell loss. After 25 years still a puzzle. *JAMA* 2006;**296**:1523–5

(Accepted 14 November 2010)