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Intracellular Concentrations of Non-Nucleoside Reverse Transcriptase Inhibitors and its Potential Role on Apoptosis in Peripheral Blood Mononuclear Cells

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Abstract

Background: Efavirenz (EFV) and nevirapine (NVP) are non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are frequently used in combination with other antiretrovirals for the treatment of HIV-infected persons. Little information is available regarding the intracellular concentrations (ICs) of EFV and NVP in peripheral blood mononuclear cells (PBMCs) and its potential role for cellular toxicity.

Methods: PBMCs from healthy adult donors were treated with or without the mean peak steady-state levels (Cmax) of EFV (12.4μM) and NVP (17.0μM) in human plasma during antiretroviral therapy multiplied by 0.5, 1.0, 2.0 and 4.0. After 48 hr treatment, ICs of EFV and NVP were measured using liquid chromatoagraphy-ion trap/mass spectrometry. The degree of apoptotic cells and mitochondrial membrane potential in PBMCs were measured by flow cytometry.

Results: The mean log ICs of x1.0 Cmax NVP in PBMCs (2.00 ± 0.23 μM) were significantly lower than the one of x1.0 Cmax EFV (2.95 ± 0.22 μM) ($P < 0.01$). Similar significant differences of mean log ICs were observed when the concentration of NNRTIs were x0.5 Cmax (1.62 ± 0.26 μM vs. 2.87 ± 0.13 μM, $P < 0.01$) and x2.0 Cmax (1.99 ± 0.39 μM vs. 3.11 ± 0.21 μM, $P < 0.01$). Furthermore, apoptotic PBMCs were lower than PBMC treated with the concentrations of NVP above the plasma Cmax observed clinically in patients as compared to those treated with comparable concentrations of EFV ($P < 0.01$).

Conclusion: These *in vitro* data suggest that ICs of NVP in PBMCs are significantly lower than ICs of EFV in PBMC and are also associated with less apoptotic PBMCs. The clinical relevance of this observation remains to be elucidated.

Keywords: Non-nucleoside reverse transcriptase inhibitors (NNRTIs); Efavirenz; Nevirapine; Apoptosis; CYP2B6; Intracellular concentrations

Introduction

Highly active antiretroviral therapy (HAART) has significantly changed the morbidity and mortality associated with HIV-1 infection [1,2,3]. A non-nucleoside reverse transcriptase inhibitor (NNRTI) combined with two or three nucleoside reverse transcriptase inhibitors (NRTIs) is recommended in the current treatment guidelines for the treatment of antiretroviral naïve patients [4]. NNRTI-based regimens are frequently the preferred choice for many patients compared to protease inhibitor (PI)-based regimens because of lower pill burdens resulting in both better compliance [5] and better lipid profiles [6,7].

Among NNRTIs, efavirenz (EFV) has been included in the majority of treatment guidelines as the preferred first-line regimen for HIV-infected adults [4] and children >3 years old in the United States and developed countries. In contrast, nevirapine (NVP) is the most commonly used NNRTI in developing countries where it is also commonly used for the prevention of mother-to-child transmission (MTCT) [8,9,10,11,12].

Most patients experience an excellent response to NNRTI-containing regimens; however, a subset of individuals responds poorly with slow virologic suppression and immunologic recovery, due to the rapid development of drug resistance [13]. Although there are other factors contributing to these different clinical responses, recent pharmacogenetic studies suggest that at least some of these variable responses to NNRTI-containing regimens are predictable based on variants in genes responsible for metabolizing and transporting antiretrovirals [14]. We previously reported that specific *CYP2B6* genetic variants were determinants of NVP pharmacokinetics (PK) and affected the immunologic recovery of HIV-1 infected children who received NVP containing HAART [15]. Children with *CYP2B6* variants had lower NVP oral clearance and demonstrated better immunologic recovery than those with the wild-type *CYP2B6* genotype. In contrast, although children with *CYP2B6* variants receiving an EFV containing HAART regimen achieved higher plasma concentrations of EFV, we failed to observe improved immunologic recovery [16].

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Intracellular concentrations (ICs) of NNRTIs are determined by a net result of passive transport, active uptake, efflux from cells, and metabolization of enzymes such as CYP3A4 and CYP2B6 in peripheral blood mononuclear cells (PBMCs). The cellular accumulation of NNRTIs may vary among individuals and measurement of ICs of NNRTIs may adequately reflect treatment efficacy and drug toxicity than measurement of plasma drug concentrations. Therefore, measuring IC of antiretrovirals is important. We hypothesized that ICs of EFV and NVP are different and the difference may affect cell survival because EFV is known to induce caspase- and mitochondrion-dependent apoptosis in PBMCs [17]. A few studies evaluated ICs of EFV and NVP in HIV-infected patients [18,19,20]; however, no study compared the differences in ICs of EFV and NVP using an *in vitro* model.

Materials and Methods

Peripheral blood mononuclear cells (PBMCs) isolation

PBMCs were obtained from healthy adult donors (n = 5, multiple ethnicities and race) using a Ficoll-Paque (GE Healthcare) density gradient according to the manufacturer's instructions.

EFV and NVP treatment of PBMCs

Isolated PBMCs were counted and incubated in 3 mL of serum free media (AIM-V, Invitrogen, Carlsbad, CA) at a concentration of 1.0 x 10^6/mL in 6-well plates (Corning, Corning, NY). EFV was purchased from Morevak Biochemicals (Brea, CA) and NVP was provided by Boehringer Ingelheim (New York, NY). Both compounds were reconstituted in 50% ethanol. The cells were treated with or without the mean peak steady-state levels (Cmax) of EFV and NVP in human plasma during antiretroviral therapy multiplied by 0.5, 1.0, 2.0 and 4.0. The cells were also incubated with vehicle (50% ethanol) with the highest concentration of ethanol (0.78%) used to reconstitute EFV for each assay. The Cmax of EFV and NVP were 12.4μM and 17.0μM, respectively. The cells were treated for 48 hr at 37°C at 5% CO_2. The assays contained cells treated with each treatment condition in duplicates and were repeated five times using different donor samples.

Measurement of intracellular concentrations of EFV and NVP in PBMCs

Treated and untreated cells were analyzed for ICs of EFV and NVP using liquid chromatoagraphy-ion trap/mass spectrometry (LC-IT/MS). Equal numbers of the cells in each treatment condition were collected from the same samples for flow cytometry and the cells were lysed in 100μL of 50% methanol and were stored at −70°C until the measurement was performed. ICs of EFV and NVP were measured by positive ion electrospray ionization mass spectrometry, after separation with high pressure liquid chromatography. The response of protonated molecular ions (m/z = 267 and 316, for NVP and EFV, respectively) in PBMC were compared to the response of NVP and EFV calibrators prepared in artificial intracellular fluid [21], after adjustment for processing efficiency via internal standardization (Figure 3). The ICs were expressed μM per 10^6 PBMC.

Apoptosis and active mitochondrial membrane potential assays by flow cytometry

Treated and untreated cells were evaluated for the degree of induction of apoptosis and mitochondrial membrane potential (Δψm) by flow cytometry. The following markers were used; Annexin-V (Benton Dickinson, Franklin Lakes, NJ), propidium iodide (PI) (Benton Dickinson), and $DiIC_1(5)$ (Invitrogen). After 48-hr, the treated and untreated cells were counted and $3x10^5$ cells per each treatment condition were assessed by flow cytometry. Briefly, cells were incubated with $DiIC_1(5)$ for 30 min with appropriate positive controls. Subsequently, the cells were stained with Annexin-V, followed by PI according to the manufactures' protocols. Stained cells were analyzed by FACS at the FACS Core Laboratory at the Veterans Affair Medical Center, San Diego. Viable cells were defined based on side and forward scatter and the cells positive for Annexin-V were defined as apoptotic cells. The cells positive for PI and Annexin-V were defined as late apoptotic cells and the proportion of late-apoptotic cells to early-apoptotic and viable cells was calculated. The cells positive for $DiIC_1(5)$ and negative for PI were defined as active Δψm cells and the proportion of the cells with active Δψm to viable cells was calculated.

Statistical analyses

Statistical analyses were performed using the SPSS 13.0 software (Chicago, IL). The Student T-test was used for comparison of numerical variables in two independent groups. The analysis of variance (ANOVA) test was used for the comparison of numerical variables in ≥3 independent groups. All P-values calculated were two-sided and a P-value of < 0.05 was considered to be statistically significant.

Results

Intracellular concentrations of EFV and NVP

The mean log ICs of NVP (mean ± S.D) at x0.5 Cmax (1.62 ± 0.26 μM) were significantly lower than those of EFV at x0.5 Cmax (2.87 ± 0.13 μM) (P < 0.01). The mean log ICs of NVP (mean ± S.D) at x1.0 Cmax and x2.0 Cmax (2.00 ± 0.23 μM and 1.99 ± 0.39 μM, respectively) were significantly lower than those of EFV at x1.0 Cmax and x2.0 Cmax (2.95 ± 0.22 μM and 3.11 ± 0.21 μM, respectively) (P < 0.01, P < 0.01, respectively) (Figure 1). These results demonstrate ICs in PBMCs

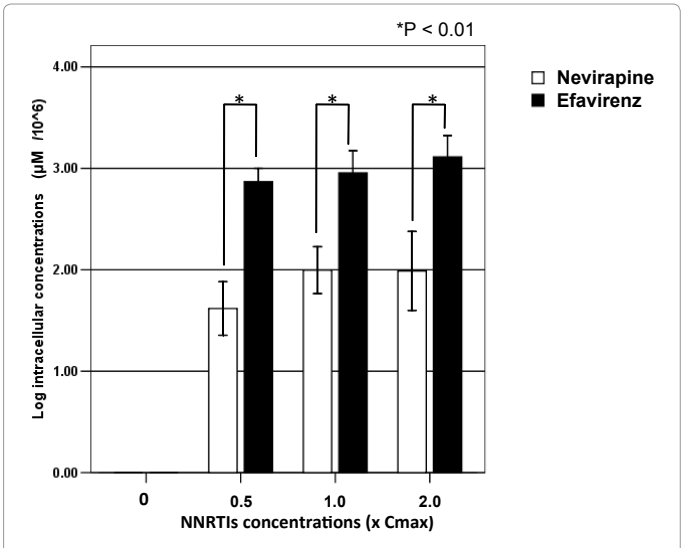

Figure 1: Intracellular concentrations of NNRTI in PBMCs treated with nevirapine and efavirenz. Open and closed bars indicate the log intracellular concentrations of nevirapine and efavirenz in PBMCs treated with different concentrations for 48 hrs, respectively. Significant differences were observed when the intracellular concentrations were compared with the same Cmax (P < 0.01). Cmax: the mean peak steady-state levels in human plasma during antiretroviral therapy.

treated with NVP were approximately one log lower compared to the cells treated with EFV.

Survival of PBMCs treated with NNRTIs

After the 48-hr incubation period, viable PBMCs in untreated groups decreased to 60% (range: 51-68%). Compared to untreated cells (1.00), the cell-survival ratios (mean ± S.D.) in PBMCs treated with 0.5x, 1.0x, 2.0x, and 4.0x Cmax of EFV were decreased in a dose dependent manner: 0.88 ± 0.18 (P = 0.15), 0.94 ± 0.16 (P = 0.24), 0.71 ± 0.28 (P = 0.06), and 0.03 ± 0.04 (P < 0.01), respectively. In contrast, the cell-survival ratios in PBMCs treated with 0.5x, 1.0x, 2.0x, and 4.0x Cmax of NVP did not change significantly: 0.93 ± 0.05 (P = 0.14), 0.92 ± 0.12 (P = 0.17), 0.84 ± 0.13 (P = 0.09), and 0.84 ± 0.13 (P = 0.09), respectively. The cell-survival ratio in the group treated with vehicle used for the maximum concentration of EFV (x2.0 Cmax) was 0.91 ± 0.12 (P = 0.87).

EFV treated PBMCs undergo greater apoptosis than NVP treated PBMCs

PBMCs treated with EFV demonstrated a higher proportion of late-apoptotic cells in a dose dependent manner compared to untreated cells (Figure 2A). The percentages (mean ± S.D.) of late-apoptotic cells in PBMCs treated with x0.5, x1.0, and x2.0 Cmax of EFV were 3.0 ± 4.1 %, 7.4 ± 3.5%, and 24.9 ± 12.9%, respectively. The apoptosis assay could not be performed when a concentration of x4.0 Cmax of EFV because at this concentration and duration of treatment no viable cells remained. NVP also exhibited a dose-dependent increase in populations of late-apoptotic cells; however, the proportion of late-apoptotic cells to early-apoptotic and viable cells was substantially less under NVP

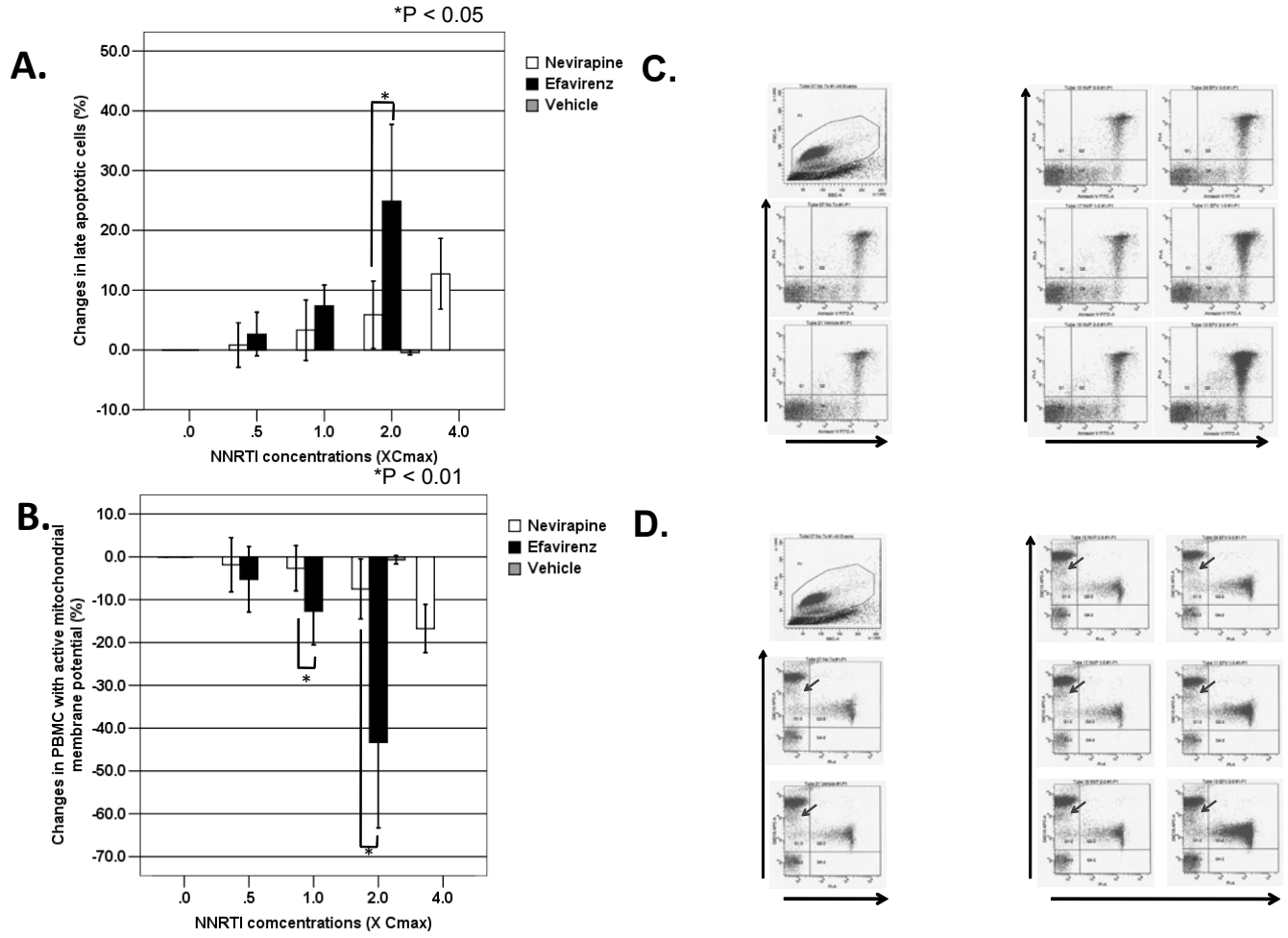

Figure 2: Induction of late apoptotic cells and mitochondrial membrane potentials in peripheral blood mononuclear cells (PBMCs) treated with nevirapine and efavirenz using different concentrations. The changes in late apoptotic cells (A) and mitochondrial membrane potential (B) from untreated cells in each treatment condition are shown with open bars (nevirapine) and closed bars (efavirenz). Significant differences were observed when the cells were treated with x2.0 Cmax of efavirenz. No data are shown in cells treated with x4.0 Cmax of efavirenz because of the low number of viable cells after 48 hr incubation. Cmax: the mean peak steady-state levels in human plasma during antiretroviral therapy. Actual FACS figures for late apoptotic cells (C) and cells with active mitochondrial membrance potential (D) are shown. (C): The viable cells were gated (Panel C-A) and the proportion of late apoptotic cells which were defined as positive for both Annexin-V and propidium iodine (PI) are indicated at the right upper corner of the panel for late apoptotic cells: untreated cells (Panel C-B), vehicle (50% ethanol concentration used for efavirenz x2.0 Cmax, Panel C-C), nevirapine treated cells with different concentrations (Panels C-D, E, F), and efavirenz treated cells with different concentrations (Panels C-G, H, I). (D): The viable cells were gated (Panel D-A) and the proportion of cells with mitochondrial membrane potential were defined as positive for DiIC1(5) and negative for PI are indicated at the left upper corner of the panel for cells with mitochondrial membrane potential: untreated cells (Panel D-B), vehicle (50% ethanol concentration used for efavirenz x2.0 Cmax, Panel D-C), nevirapine treated cells with different concentrations (Panels D-D, E, F), and efavirenz treated cells with different concentrations (Panels D-G, H, I). Cmax: the mean peak steady-state levels in human plasma during antiretroviral therapy. PI: propidium iodine.

treatment when compared to EFV treatment. The percentages (mean ± S.D.) of late-apoptotic cells in PBMCs treated with x0.5, x1.0, x2.0, x4.0 Cmax of NVP were 0.8 ± 4.5%, 3.3 ± 5.0%, 5.9 ± 5.7%, and 12.5 ± 5.8%, respectively. When equal concentrations of EFV and NVP were compared, no significant differences were observed at x0.5 Cmax (P = 0.55) and x1.0 Cmax (P = 0.36), but a significant difference between EFV and NVP was observed at x2.0 Cmax (P = 0.02).

The active mitochondrial membrane potential of PBMCs is reduced to a greater extent with exposure to EFV compared to NVP

Active Δψm exhibited trends parallel to the data on apoptosis. PBMCs treated with x0.5, x1.0, and x2.0 Cmax of EFV all showed significant decreases in populations of cells with active Δψm in a dose dependent manner (Figure 2B). No data were obtained from cells treated with x4.0 Cmax of EFV as no viable cells remained. A similar dose-dependent decrease in cells with uncompromised Δψm was observed in cells treated with NVP, although to a far lesser extent

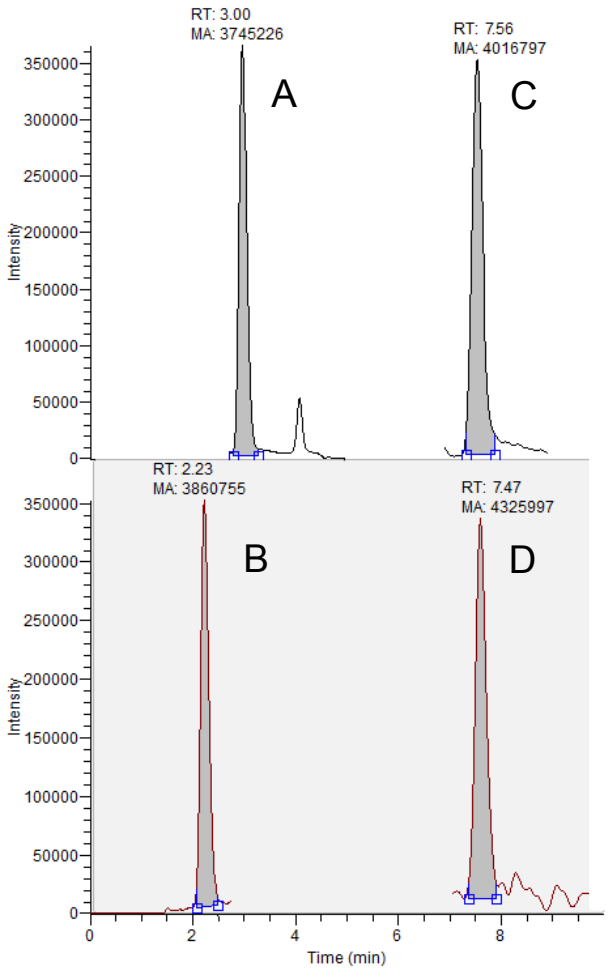

Figure 3: Mass spectrometry (MS)/MS response of peripheral blood mononuclear cell extract with 50 ng nevirapine and efavirenz, including their respective internal standards: The two left most peaks illustrate the MS/MS response of nevirapine (m/z = 267.4 > 267.4, Rt = 2.23, C) and its internal standard (BIRH-414) (m/z = 255 > 255, Rt = 3.00, A). The right most peaks represent the response of efavirenz (m/z = 316 > 272, Rt = 7.56, B) and its internal standard (d4-EFV) (m/z = 320 > 276, Rt = 7.47, D).

compared those treated with EFV. No differences were observed when the cells were treated with x0.5 Cmax of EFV and NVP (P = 0.84), but significant differences were observed when the cells were treated with x1.0 Cmax (P < 0.01) and x2.0 Cmax of EFV and NVP (P < 0.01).

Discussion

In the current study, we have shown that ICs of NVP in PBMCs were approximately one-log lower than ICs of EFV when the PBMCs were treated with different concentrations of NVP and EFV based on the mean peak steady-state levels. Furthermore, the induction of apoptosis of PBMCs is lower in PBMCs treated with concentrations of NVP above the Cmax as compared to those treated with comparable concentrations of EFV. These results suggest that differences in ICs of NVP and EFV may contribute to different cell survival in PBMCs treated with NVP and EFV.

Similar IC differences between EFV and NVP have also been demonstrated *in vivo* [20]. Rotger et al. [20] demonstrated that the IC of EFV was higher in than IC of NVP in HIV-infected adults and was affected by *CYP2B6-G516T* genotype; however, no direct comparison of the IC between EFV and NVP was performed. A number of potential mechanisms may account for the observation of a difference of ICs between NVP and EFV. First, although a clear mechanism is unknown, the degree of passive transport and active uptake may be different between NVP and EFV. Second, a role of the ATP-binding cassette, sub-family B, member 1 (*ABCB1*) needs to be considered. ABCB1 is a trans-membrane protein that actively transports many substrates from the cytoplasm to the extracellular space and is therefore a principle determinant of the pharmacokinetics of many drugs, including protease inhibitors [22]. Genetic variants in the *ABCB1* gene have been reported to be responsible for variable protease inhibitors pharmacokinetics in children [23] and adults [24]. Although neither NVP or EFV are known substrates of ABCB1 [25], several studies have shown clinical associations between *ABCB1* genotypes and NNRTIs pharmacokinetics (including EFV) [24], virologic outcomes in patients receiving EFV containing regimens,[26] and the risk of NVP-induced hepatotoxicity [27,28]. Furthermore, ICs of NVP inversely correlate with ABCB1 expression on PBMCs in subjects receiving NVP [19]. In contrast, Janneh and colleagues found that ABCB1 expression has no effect on the accumulation of EFV and NVP, and suggest that some an important role for other factors including MRP1/2 expression, lipophilicity and SLCO-like transporters [29] .

In the current studies, we used the mean peak steady-state levels (Cmax) of NVP and EFV in human plasma during antiretroviral therapy multiplied by 0.5, 1.0, 2.0 and 4.0 Cmax to reflect the actual concentrations of EFV and NVP *in vivo*. The actual x1.0 Cmax of EFV (12.4μM) was lower than x1.0 Cmax of NVP (17.0μM), demonstrating that the differences observed in the current study did not result simply from the differences of concentrations of both drugs.

Although our data demonstrate that ICs of NVP are lower than that of EFV and induce less apoptotic cells compared to EFV in PBMC when the cells are treated with close to the mean-peak steady state concentrations, no clinical data, to our knowledge, have identified differences in immunologic recovery between NVP and EFV regimens [30,31]; therefore, the clinical relevance of our findings remains uncertain. Other factors that may alter the ICs of NVP and EFV will need to be considered including hepatic CYP2B6 genotypes [32] and metabolization of each drug by CYP2B6 and CYP3A4 in PBMC [33].

Because we were not able to find a direct correlation between ICs of NNRTIs and cell survival or induction of apoptosis, these factors may contribute our findings.

In addition, although our observations showed significant differences in late apoptotic cells between NVP and EFV during the 48-hr incubation period, *in vivo* data demonstrated that no differences were observed in the extent of apoptosis or in $\Delta\psi$ between EFV or NVP treatments in lymphocytes from HIV-1 infected patients [34]. In this study, the duration of the NVP regimen correlated with apoptosis and $\Delta\psi m$ in lymphocytes from HIV-1 infected patients. Therefore, the cumulative effects of NVP on lymphocytes may account for the discrepancy between *in vitro* and *in vivo* studies.

In conclusion, PBMCs treated with NVP induced significantly less apoptosis than EFV when treated with higher than the mean peak steady-state levels of NNRTI in human plasma during antiretroviral therapy. The clinical relevance of this observation remains to be elucidated.

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References

1. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, et al. (2003) Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet 362: 22-29.
2. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, et al. (1998) Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 338: 853-860.
3. Gortmaker SL, Hughes M, Cervia J, Brady M, Johnson GM, et al. (2001) Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 345: 1522-1528.
4. Hammer SM, Saag MS, Schechter M, Montaner JS, Schooley RT, et al. (2006) Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. JAMA 296: 827-843.
5. Trotta MP, Ammassari A, Cozzi-Lepri A, Zaccarelli M, Castelli F, et al. (2003) Adherence to highly active antiretroviral therapy is better in patients receiving non-nucleoside reverse transcriptase inhibitor-containing regimens than in those receiving protease inhibitor-containing regimens. AIDS 17: 1099-1102.
6. McComsey G, Bhumbra N, Ma JF, Rathore M, Alvarez A (2003) Impact of protease inhibitor substitution with efavirenz in HIV-infected children: results of the First Pediatric Switch Study. Pediatrics 111: e275-281.
7. Young J, Weber R, Rickenbach M, Furrer H, Bernasconi E, et al. (2005) Lipid profiles for antiretroviral-naive patients starting PI- and NNRTI-based therapy in the Swiss HIV cohort study. Antivir Ther 10: 585-591.
8. Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, et al. (1999) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet 354: 795-802.
9. Jackson JB, Musoke P, Fleming T, Guay LA, Bagenda D, et al. (2003) Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. Lancet 362: 859-868.
10. Ayouba A, Tene G, Cunin P, Foupouapouognigni Y, Menu E, et al. (2003) Low rate of mother-to-child transmission of HIV-1 after nevirapine intervention in a pilot public health program in Yaounde, Cameroon. J Acquir Immune Defic Syndr 34: 274-280.
11. Taha TE, Kumwenda NI, Gibbons A, Broadhead RL, Fiscus S, et al. (2003) Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: NVAZ randomised clinical trial. Lancet 362: 1171-1177.
12. Taha TE, Kumwenda NI, Hoover DR, Fiscus SA, Kafulafula G, et al. (2004) Nevirapine and zidovudine at birth to reduce perinatal transmission of HIV in an African setting: a randomized controlled trial. JAMA 292: 202-209.
13. Kozal MJ, Hullsiek KH, Macarthur RD, Berg-Wolf M, Peng G, et al. (2007) The Incidence of HIV drug resistance and its impact on progression of HIV disease among antiretroviral-naive participants started on three different antiretroviral therapy strategies. HIV Clin Trials 8: 357-370.
14. Owen A, Pirmohamed M, Khoo SH, Back DJ (2006) Pharmacogenetics of HIV therapy. Pharmacogenet Genomics 16: 693-703.
15. Saitoh A, Sarles E, Capparelli E, Aweeka F, Kovacs A, et al. (2007) CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. AIDS 21: 2191-2199.
16. Saitoh A, Fletcher CV, Brundage R, Alvero C, Fenton T, et al. (2007) Efavirenz pharmacokinetics in HIV-1-infected children are associated with CYP2B6-G516T polymorphism. J Acquir Immune Defic Syndr 45: 280-285.
17. Pilon AA, Lum JJ, Sanchez-Dardon J, Phenix BN, Douglas R, et al. (2002) Induction of apoptosis by a nonnucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitor. Antimicrob Agents Chemother 46: 2687-2691.
18. Almond LM, Edirisinghe D, Dalton M, Bonington A, Back DJ, et al. (2005) Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus-infected individuals. Clin Pharmacol Ther 78: 132-142.
19. Almond LM, Hoggard PG, Edirisinghe D, Khoo SH, Back DJ (2005) Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. J Antimicrob Chemother 56: 738-744.
20. Rotger M, Colombo S, Furrer H, Bleiber G, Buclin T, et al. (2005) Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 15: 1-5.
21. Zhou YD, Acker CD, Netoff TI, Sen K, White JA (2005) Increasing Ca2+ transients by broadening postsynaptic action potentials enhances timing-dependent synaptic depression. Proc Natl Acad Sci U S A 102: 19121-19125.
22. Lee CG, Gottesman MM, Cardarelli CO, Ramachandra M, Jeang KT, et al. (1998) HIV-1 protease inhibitors are substrates for the MDR1 multidrug transporter. Biochemistry 37: 3594-3601.
23. Saitoh A, Singh KK, Powell CA, Fenton T, Fletcher CV, et al. (2005) An MDR1-3435 variant is associated with higher plasma nelfinavir levels and more rapid virologic response in HIV-1 infected children. Aids 19: 371-380.
24. Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, et al. (2002) Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. Lancet 359: 30-36.
25. Stormer E, von Moltke LL, Perloff MD, Greenblatt DJ (2002) Differential modulation of P-glycoprotein expression and activity by non-nucleoside HIV-1 reverse transcriptase inhibitors in cell culture. Pharm Res 19: 1038-1045.
26. Haas DW, Smeaton LM, Shafer RW, Robbins GK, Morse GD, et al. (2005) Pharmacogenetics of Long-Term Responses to Antiretroviral Regimens Containing Efavirenz and/or Nelfinavir: An Adult AIDS Clinical Trials Group Study. J Infect Dis 192: 1931-1942.
27. Haas DW, Bartlett JA, Andersen JW, Sanne I, Wilkinson GR, et al. (2006) Pharmacogenetics of nevirapine-associated hepatotoxicity: an Adult AIDS Clinical Trials Group collaboration. Clin Infect Dis 43: 783-786.

28. Ritchie MD, Haas DW, Motsinger AA, Donahue JP, Erdem H, et al. (2006) Drug transporter and metabolizing enzyme gene variants and nonnucleoside reverse-transcriptase inhibitor hepatotoxicity. Clin Infect Dis 43: 779-782.

29. Janneh O, Chandler B, Hartkoorn R, Kwan WS, Jenkinson C, et al. (2009) Intracellular accumulation of efavirenz and nevirapine is independent of P-glycoprotein activity in cultured CD4 T cells and primary human lymphocytes. J Antimicrob Chemother 64: 1002-1007.

30. Bannister WP, Ruiz L, Cozzi-Lepri A, Mocroft A, Kirk O, et al. (2008) Comparison of genotypic resistance profiles and virological response between patients starting nevirapine and efavirenz in EuroSIDA. AIDS 22: 367-376.

31. Manosuthi W, Sungkanuparph S, Vibhagool A, Rattanasiri S, Thakkinstian A (2004) Nevirapine- versus efavirenz-based highly active antiretroviral therapy regimens in antiretroviral-naive patients with advanced HIV infection. HIV Med 5: 105-109.

32. Elens L, Vandercam B, Yombi JC, Lison D, Wallemacq P, et al. (2010) Influence of host genetic factors on efavirenz plasma and intracellular pharmacokinetics in HIV-1-infected patients. Pharmacogenomics 11: 1223-1234.

33. Liptrott NJ, Penny M, Bray PG, Sathish J, Khoo SH, et al. (2009) The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes and chemokine receptors in human PBMC. Br J Pharmacol 156: 497-508.

34. Karamchand L, Dawood H, Chuturgoon AA (2008) Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. J Acquir Immune Defic Syndr 48: 381-388.