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Short Communication

# *In silico* study supports the efficacy of a reduced dose regimen for stavudine

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ABSTRACT

Stavudine (d4T) is used extensively as part of HAART in resource poor settings, despite its toxicities. The revised WHO guidelines specify replacement of d4T with less toxic but more expensive drugs when feasible, and that d4T doses be standardized to 30 mg twice daily (bid) (irrespective of body-weight), from the approved 40 mg bid in adults (body-weight $\geqslant$60 kg). Therefore, an *in silico* population pharmacokinetic and biochemical model was utilized to compare relative efficacies of the two doses in humans. Assessment of predicted quartile ranges of simulated concentrations of the triphosphate of d4T suggested sufficient trough concentrations to inhibit wild type HIV-1 reverse transcriptase at the reduced dose, lending support to the revised WHO recommendations.

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Nucleoside reverse transcriptase inhibitors (NRTIs) remain the backbone of highly active antiretroviral therapy (HAART), in combination with protease (PI), non-nucleoside reverse transcriptase (NNRTI), integrase, or entry/fusion inhibitors. NRTIs are essentially prodrugs which undergo intracellular phosphorylation to their active triphosphate forms (NRTI-TP), which exert antiviral activity primarily *via* competitive inhibition of HIV-1 reverse transcriptase (HIV-1 RT) (Schinazi et al., 2006). Most HIV-1-infected persons live in poor and middle-income countries, and rely on the availability of inexpensive generic fixed-dose combinations of HAART (McCutchan, 2009; van Griensven et al., 2009). The low cost of stavudine (d4T) has enabled the scale up of HAART in these settings. According to the WHO, 56% of adults on HAART in low- and middle-income settings received d4T as part of their HIV regimens (WHO, 2010; Casiro, 2011). This figure is even higher in Sub-Saharan Africa where 80% of infected individuals received d4T between 2005 and 2006, compared with 66% in Asia and 15% in South America (WHO, 2009; Keiser et al., 2008). Several fixed dose combinations are currently marketed in Africa, usually in combination with 150 mg lamivudine and 200 mg nevirapine. The original formulations contained 40 mg d4T (e.g., GPO-VIR S 40™, Thai Government Pharmaceutical Organization; Triomune-40™, Cipla Ltd.; Virolans™ capsules, d4T 40 mg version, Ranbaxy Laboratories Ltd.). However, d4T is associated with debilitating long term side effects, associated with mitochondrial dysfunction, which include hyperlactemia, lipodystrophy, neuropathies, and lactic acidosis, some of which are irreversible (McComsey and Lonergan, 2004; van Griensven et al., 2009). Randomized studies on first-line HAART in first-world and in resource limited settings, indicated that zidovudine (ZDV) and tenofovir had similar efficacy and produced fewer metabolic side effects compared with d4T (Bygrave et al., 2011; Dube et al., 2005, 2007; Gallant et al., 2004; Mercier et al., 2009; Pujari et al., 2005). These toxicities prompted the World health Organization (WHO) in 2009, to recommend that countries phase out d4T (WHO, 2009; Wainberg, 2009). Although d4T (and ZDV) is off patent and is manufactured as a low cost generic medication in combination for use in 80 poor countries (ViiV Healthcare Web site), and tenofovir disoproxil fumarate is being offered by Gilead, its manufacturer, at a tiered discounted price in low- and middle-income countries (Gilead Web site), prices still remain beyond the reach of the poorest countries. Given the magnitude of the infected population, together with economic and other constraints, the WHO revised its recommendation to specify that "safer but currently more expensive first-line ARTs should be progressively introduced as currently they may not be feasible or affordable in many high-burden settings with low coverage, less developed health systems, limited laboratory capacity, finite budgets and competing health priorities" The recommendations also specify that a reduced dosage of 30 mg twice daily (bid) should be administered to all individuals on d4T in an attempt to reduce side effects (WHO, 2010).

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Pilot and retrospective studies suggested that reduction in d4T dosage from 40 to 30 mg twice daily (bid), in individuals weighing less than 60 kg and possibly heavier individuals as well, may decrease the incidence of side effects, without compromising antiviral efficacy (Ait-Mohand et al., 2008; Hoffmann et al., 2009; Karara et al., 2010; Pedrol et al., 2007; Sanchez-Conde et al., 2005). However, d4T dose reduction to control toxicity remains controversial. Domingo et al., 2010, reported a statistically significant ($p = 0.013$) higher median stavudine triphosphate (d4T-TP) content in the lymphocyte peripheral blood mononuclear (PBM) cells of 17 individuals with HAART associated lipodystrophy syndrome (HALS) (20.6 femtomoles/$10^6$ cells in individuals with HALS, interquartile range (IQR) = 14.90–26.92), compared to 16 individuals without HALS. (13.85 fmol/$10^6$ cells, IQR = 8.65–20.15) (Domingo et al., 2010). However, it is possible that the differences in d4T-TP levels between the two groups would have been even greater had d4T-TP measurements been normalized with the fraction of dividing cells (d4T is primarily phosphorylated in dividing lymphocytes), and had blood sampling times been standardized according to the time of the last d4T administration. Another recent study reported a lower risk of developing lipoatrophy in subjects who received 30 mg d4T exclusively (regardless of body weight, as suggested by WHO 2007 guidelines) since the start of treatment ($n = 69$), than in individuals who received weight adjusted d4T doses (30 mg if $\leqslant$60 kg, and 40 mg otherwise, patients treated before WHO 2007 guidelines) ($n = 64$) since treatment initiation. Furthermore, the odds ratio for the 30 mg bid only cohort was similar to a control group treated with AZT ($n = 110$), a drug which is not associated with lipoatrophy (Cournil et al., 2010). In contrast, a study involving 47 HIV-infected individuals did not find a correlation between lipodystrophy and d4T plasma exposures (AUC), with some individuals demonstrating this side effect even at a 20 mg bid dose (Sinxadi et al., 2010). The revised WHO guideline (WHO, 2010) now recommended that where d4T use is continued, it be administered at 30 mg BID for all individuals, irrespective of body weight. Based on the expectation of reduced toxicity with maintenance of adequate viral inhibition, fixed dose combination formulations containing d4T at the reduced 30 mg dosage with the standard lamivudine and nevirapine dosages are presently in use (e.g., Stavex-30 LN).

Population pharmacokinetic and pharmacodynamic simulations are useful for consolidating all available drug information into a usable form for the design of clinical trials of antiretroviral agents. These simulations may allow detailed analysis of dosage regimens before actual studies are conducted. For example, based on predicted antiviral response, Rosario et al. utilized clinical trial simulations to streamline the phase 2 development of the CCR5 receptor blocking agent maraviroc (Rosario et al., 2005). Furthermore, our group developed simulation models for NRTI to predict the antiviral response of lamivudine, and to suggest a reduced dosage regimen for zidovudine (Hurwitz et al., 2007, 2008). The pharmacological and biochemical parameters of d4T necessary for modeling, including population pharmacokinetics, cellular phosphorylation in activated PBM cells (the major target for HIV-1 infection and replication), and the potency of inhibition of inhibition of the HIV-1 RT reverse transcription enzyme by d4T-TP have been studied (Becher et al., 2004; Panhard et al., 2007; Ueno and Mitsuya, 1997). Therefore, it was considered feasible to merge these data *in silico* using mechanistic data, and to conduct a virtual dosing study to assess whether d4T is expected to maintain efficacy at the 30 mg bid dose, as suggested by the clinical pilot studies.

Becher et al. reported a linear correlation ($r^2 = 0.45$), between the accumulation of d4T-TP in the PBM cells of subjects and d4T plasma concentrations of individuals taking d4T in a HAART cocktail. However, d4T is a thymidine analog and is phosphorylated primarily in activated ($CD4^+$) lymphocytes (Jacobsson et al., 1995). The proportion of activated PBM cells varies between infected individuals and is not usually reported in clinical studies, making it difficult to assess the linearity of d4T-TP *versus* extracellular d4T using pooled *in vivo* data from pooled individuals, each with differing activated lymphocyte fractions. Therefore the linearity between d4T and cellular accumulation of d4T-TP was tested in phytohaemagglutinin (PHA) stimulated PBM cells in cell culture, in which the degree of activation is more uniform (Hernandez-Santiago et al., 2005, 2007a, b). The concentrations measured *in vitro* were then be compared to average concentrations in humans after normalizing using 8% as an average estimate of dividing lymphocytes measured in infected individuals (Mohri et al., 2001). Since maximal d4T plasma concentrations ($C_{max}$) in individuals receiving 40 mg bid rarely exceed 7 μM, are reached within 0.5–1 h ($T_{max}$), and subsequently decay with a plasma half-life ($t_{1/2}$) of 1 to 1.5 h (Horton et al., 1995), cells were incubated with 0.5–30 μM d4T in triplicate for 4 h before rinsing in ice cold phosphate buffered saline and extracted with ice cold 60% methanol in water and drying under a gently filtered air flow. The extracted d4T-TP was redissolved in mobile phase and assayed by LC-MS/MS (Fromentin et al., 2009; Hernandez-Santiago et al., 2007a). The cellular accumulation of d4T-TP increased linearly with d4T concentration at clinically relevant concentrations ($r^2$ between 0.5 and 10 μM = 0.99), but appeared to plateau at >10 μM (Fig. 1). d4T-TP concentrations in the linear range of the curve (0.05–3.4 μM) included maximal cellular concentrations observed in the clinic after adjusting for an average of 8% activated lymphocytes in HIV-infected humans (Fig. 2) (Becher et al., 2004). Therefore, saturation of phosphorylation is not a concern at clinically relevant doses. This supported our model assumption that the accumulation rate of d4T-TP in dividing PBM cells in humans was proportional to extracellular d4T concentrations at clinically relevant doses (below).

An *in vivo* population pharmacokinetic and cellular biochemical model was used to simulate plasma d4T and cellular d4T-TP concentrations *versus* time and dose for HIV-1 infected individuals administered d4T, using the ADVAN9 differential equation routine of NONMEM (6.2, ICON Development Solutions, Ellicott City, MD), together with PLTtools (3.0, PLTsoft, San Francisco, CA). The model assumed that the nucleoside analog d4T achieves rapid equilibration between extra- and intra- cellular concentrations. This may be justified, since nucleoside analog are substrates for rapidly

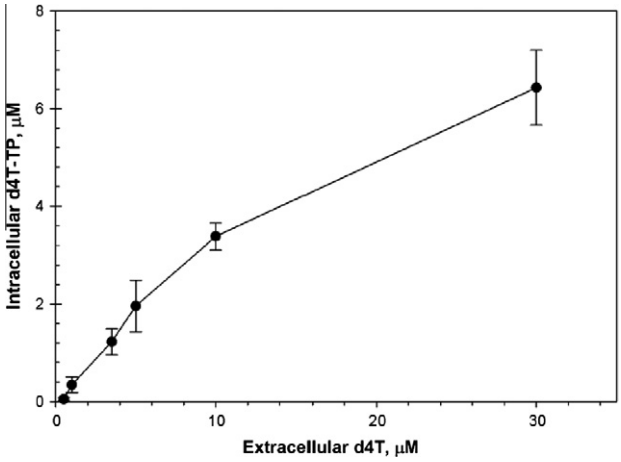


**Fig. 1.** Concentrations of d4T-TP (μM) in activated human PBM cells following a 4 h exposure to varying concentrations of d4T (•). Data shown are mean +/− SD. Plasma concentrations in the clinic are usually in the range of 0.5 to 10 μM. There was good linearity up to the highest clinical concentration range of 7 μM ($r^2 > 0.99$).