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Changes in metabolic toxicity after switching from stavudine/didanosine to tenofovir/lamivudine—a Staccato trial substudy

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***Objectives*: Stavudine is widely used in Thailand and is associated with mitochondrial toxicity. Here, we evaluated the effect of switching from stavudine/didanosine to tenofovir/lamivudine on measures of metabolic and mitochondrial toxicity in Thai patients.**

***Methods*: Thirty-five Thai patients with full HIV RNA suppression were switched from stavudine/didanosine to tenofovir/lamivudine while receiving saquinavir/ritonavir 1600/100 mg once daily. Patients were assessed at the time of switch and 24 and 48 weeks after for lipids, liver enzymes, lactate, mitochondrial DNA content and limb/total fat mass by dual energy X-ray absorptiometry (DEXA) scanning.**

***Results*: Forty-eight weeks after the switch, there were significant reductions in lipids and lactate, but no change in liver enzymes. There was reversal of lipoatrophy, as shown by rises in limb fat mass (+0.38 kg, $P = 0.006$) and total fat mass (+0.69 kg, $P = 0.02$) on DEXA scan. Patients perceived weight improvement, but did not report reversal of lipoatrophy of individual body parts. The mitochondrial DNA/nuclear DNA ratio rose (+1.06, $P < 0.0001$).**

***Conclusions*: After the nucleoside reverse transcriptase inhibitor switch, reversal of mitochondrial toxicity was consistent with switch studies of mainly Caucasian patients, although the peripheral mononuclear cell mitochondrial DNA rise exceeded previous reports.**

Keywords: mitochondrial toxicity, NRTIs, Thailand

Introduction

As highly active antiretroviral therapy (HAART) is often life long, minimizing toxicity is a priority. Lipoatrophy, a condition characterized by the atrophy of adipose tissue on extremities and the face, along with accumulation of visceral fat, develops in many patients treated with a combination of protease inhibitor and nucleoside reverse transcriptase inhibitors (NRTIs).[1] Mitochondrial toxicity accompanied by decreased mitochondrial DNA, increased serum lactate and fat atrophy has been associated with NRTIs in general and stavudine in particular. Adipose tissue mitochondrial DNA depletion is an important determinant of stavudine-induced lipoatrophy pathogenesis. These changes are partially reversed when stavudine is stopped.[2,3] However,

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this relationship has not been established for peripheral blood mononuclear cell (PBMC) mitochondrial DNA, where it appears that didanosine is the dominant treatment-related determinant of mitochondrial DNA depletion.[4–6] Mitochondrial DNA changes in PBMCs do not appear to predict the mitochondrial DNA changes in tissue[5,7] and can vary regardless of NRTI use.[4]

In randomized Phase 3 trials, first-line treatment with tenofovir leads to lower rates of clinically diagnosed lipoatrophy and lipid elevations, relative to either stavudine[8] or zidovudine.[9] Previous studies have shown improvements in lipoatrophy and lipid parameters after a switch to either tenofovir or abacavir.[10] In the A5005s study, a sub-study of ACTG 384, patients given first-line stavudine/didanosine-based HAART showed greater rises in lipid parameters and a higher incidence of lipoatrophy, relative to those given zidovudine/lamivudine.[11] There is also evidence that higher stavudine doses may increase the incidence of lipoatrophy.[12]

Staccato was a randomized trial of intermittent versus continuous antiretroviral treatment. Prior to randomization, antiretroviral-naive Thai patients with CD4 counts of 200–350 cells/mm^3 were given first-line treatment with stavudine/didanosine (stavudine 30 or 40 mg, didanosine 250 or 400 mg based on weight below or above 60 kg) plus saquinavir/ritonavir (1600/100 mg once daily), until HIV RNA was suppressed below 50 copies/mL and CD4 count was above 350 cells/mm^3. They were then randomized to either continuous or interrupted treatment.[13,14] During the Staccato trial, the NRTI backbone for Thai patients was switched from stavudine/didanosine to tenofovir/lamivudine according to new treatment guidelines, although saquinavir/ritonavir treatment was maintained. This offered the opportunity to study the effect of this change on measures of metabolic and mitochondrial toxicity.

Methods

This substudy included 35 patients in the continuous treatment arm, who were consecutively recruited at one Thai centre. We measured changes in mitochondrial and metabolic toxicity before and after switching from stavudine/didanosine to tenofovir/lamivudine.

Patients were assessed at the time of switch from stavudine/didanosine to tenofovir/lamivudine (week 0) and at 24 and 48 weeks after the switch. The patients were evaluated for lipids [triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides], liver enzymes [alanine transaminase (ALT)] and lactate. At weeks 0, 24 and 48, patients completed a lipodystrophy questionnaire, where they answered questions about change in various body parts in the previous 6 months, on a 7-point scale with the mid value representing normal and the values on either side representing mildly, moderately or much thinner or fatter. Patients were also asked whether their weight had increased, decreased or stayed the same compared with the previous 6 months. Mitochondrial DNA was measured from PBMCs in all 35 patients, as described previously[15,16] and in blinded fashion. In addition, lipoatrophy was assessed by dual energy X-ray absorptiometry (DEXA) scanning at weeks 0 and 48 in 22 patients. During the trial, two patients became pregnant. One patient, with an approximate break from tenofovir of 2 months with substitution to zidovudine until premature termination of pregnancy, and then switch back to tenofovir, was kept in the analysis. The other patient stopped tenofovir with zidovudine substitution after week 24, so week 48 results were deleted from the data set.

Differences over time were assessed by the measured change in scores from baseline to week 24 and week 48. Results are expressed as median [interquartile range (IQR)]. For analysis of the lipodystrophy questionnaire, we attributed a score to each answer an individual patient made and summed the difference from scores from baseline to week 48. A formal comparison of the cumulative change in score from baseline was made using the Wilcoxon rank sum test.

The Staccato protocol was accepted by the local Ethics Committees from the different participating centres, and written informed consent was obtained from each participant. The Staccato study is registered at ClinicalTrials.gov with the identifier NCT00113126.

Results

Of the 35 patients in the substudy, 22 (63%) were female and 13 (37%) were male. The median age was 34 (IQR 31–39) years and the median weight was 55 (IQR 49–61.5) kg. The median baseline CD4 count was 548 (IQR 483–682) cells/mm^3. The median time on stavudine/didanosine was 86 (IQR 69–182) weeks. The baseline metabolic parameters are shown in Table 1. The median values of triglycerides, cholesterol and HDL were mildly abnormal. The median mitochondrial DNA/nuclear DNA ratio was 1.22 (IQR 0.85–1.55), and the median limb and total fat masses were 5.1 (IQR 2.7–7.2) and 10.2 (IQR 8.2–13.7) kg, respectively. Twenty-eight patients had an assessment of lipodystrophy at the time of switching to tenofovir, and, of these, 15 (54%) had lipodystrophy. Nine (26%) patients experienced peripheral neuropathy at the time of starting tenofovir. The baseline characteristics (gender, age, weight, CD4 and lipids) of the patients in this substudy did not differ significantly from the other 94 patients in Staccato at our centre.

During the 48 weeks after the switch to tenofovir/lamivudine, the median CD4 count rose by 40 cells ($P = 0.009$). HIV RNA levels were suppressed below 50 copies/mL in 94% of the patients at baseline, and this suppression rate was maintained at week 48.

After the switch from stavudine/didanosine to tenofovir/lamivudine, the mitochondrial DNA content (expressed as the relative mitochondrial DNA/nuclear DNA ratio) rose significantly to a larger extent than reported in other studies involving

Table 1. Median metabolic parameters before switching from stavudine/didanosine to tenofovir/lamivudine

Baseline variables	Median (IQR)
Triglycerides, mg/dL	147 (97–327)
Cholesterol, mg/dL	212 (192–233)
HDL, mg/dL	59 (48–70)
LDL, mg/dL	112 (93–127)
ALT, U/L	28 (19–50)
Lactate, mmol/L	1.5 (1.1–2.3)
Mitochondrial DNA/nuclear DNA ratio	1.22 (0.85–1.55)
Limb fat mass, kg	5.1 (2.7–7.2)
Total fat mass, kg	10.2 (8.2–13.7)

The upper limits of normal are as follows: triglycerides (140 mg/dL), cholesterol (250 mg/dL), LDL (160 mg/dL), ALT (31 mg/dL), lactate (2.4 mmol/L). HDL below 75 mg/dL is abnormal.

patients who either discontinued[15] or changed dideoxy NRTI to tenofovir.[17] There were significant reductions in the levels of the lipid parameters (triglycerides, total cholesterol, HDL and LDL) measured at both weeks 24 and 48 (Table 2), with the exception of LDL at week 48. Levels of ALT did not change significantly during the 48 week trial interval, but there were significant reductions in lactate levels. There was evidence of reversal of lipoatrophy on DEXA scan, as shown by significant rises in limb fat mass (+0.38 kg, $P = 0.006$) and total fat mass (+0.69 kg, $P = 0.02$) (Table 2). Patients' own subjective assessment by lipodystrophy questionnaire showed the following median (IQR) cumulative change scores: face=0 (−1 to 1), abdomen=0 (0 to 1), legs=0 (0 to 0), arms=0 (−1 to 0), thighs=0 (0 to 0) and back and base of the neck=0 (0 to 0). None of these changes was significant. The median cumulative change in patient perception of overall weight was 1 (0–3) ($P < 0.0001$). There was no significant correlation between changes in mitochondrial DNA and improvement of lipid concentrations or fat mass at week 48.

Discussion

Our study showed that switching from stavudine/didanosine to tenofovir/lamivudine resulted in significant reductions in lipids and lactate and rises in limb and total fat masses and mitochondrial DNA/nuclear DNA ratio. Patients perceived an overall weight gain but not improvement of lipoatrophy.

Table 2. Changes in laboratory values and DEXA scan measurements from baseline to weeks 24 and 48

Week	Median change	IQR	*P* value
Triglycerides (mg/dL)			
24	−19	−60, +4	0.014
48	−25	−64, +4	0.006
Cholesterol (mg/dL)			
24	−17	−43, −4	<0.0001
48	−9.5	−21, 3	0.027
HDL (mg/dL)			
24	−11	−16, −3	<0.0001
48	−3.5	−8, 2	0.02
LDL (mg/dL)			
24	−11	−38, 1	0.0004
48	−7.5	−19, 7	0.13
ALT (U/L)			
24	−7	−22, 9	0.06
48	−1.5	−1.5, 7	0.14
Lactate (mmol/L)			
24	−0.5	−1.2, −0.1	<0.0001
48	−0.45	−1.2, −0.2	<0.0001
Mitochondrial DNA/nuclear DNA ratio			
24	+1.5	0.84, 1.78	<0.0001
48	+1.06	0.67, 1.57	<0.0001
Limb fat mass (kg)			
48	+0.38	−0.13, 1.33	0.006
Total fat mass (kg)			
48	+0.69	−0.28, 2.1	0.02

The improvements in lipids and fat mass in our study are similar to those seen in the GS903, MITOX and RAVE studies of the switch from stavudine to either tenofovir or abacavir.[10,18,19] It is not surprising that the patients did not perceive a significant improvement in body shape as clinical lipoatrophy resolves slowly following treatment modification. The rise in PBMC mitochondrial DNA after stopping stavudine/didanosine suggests that this regimen caused mitochondrial depletion in PBMC. Whether this effect was due to stavudine or didanosine is unclear. Cherry *et al.*[5] found evidence for didanosine-associated PBMC mitochondrial DNA depletion, but not for stavudine. The rise in mitochondrial DNA was not correlated with other metabolic improvements in our study supporting the existing evidence which questions the reliability of PBMC mitochondrial DNA as a marker of lipoatrophy risk.[4,5]

HAART regimens containing stavudine are still widely used in Southeast Asia and sub-Saharan Africa, owing to low cost and availability of fixed-dose combinations.[20] Minimum costs of HAART in least developed countries are $339 per person-year for the combination of tenofovir, lamivudine and efavirenz versus $139 per person-year for co-formulated stavudine, lamivudine and nevirapine;[21] there is a similar price difference in Thailand. Stavudine is still used in preference to tenofovir in Thailand as first-line treatment, due to cost pressures. Simply starting a first-line regimen with tenofovir would lessen metabolic and mitochondrial toxicity. Given the modest improvement in our study, however, it is uncertain whether avoiding stavudine altogether is the best practice in settings where resources are limited if fewer people would be treated owing to the high cost of alternative NRTIs. Other strategies would be early switch from stavudine to tenofovir when there are any signs or symptoms of mitochondrial toxicity or after 1–2 years on stavudine.

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Transparency declarations

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