COMPARISON OF THAI GOVERNMENT MANUFACTURED TENOFOVIR (TENOFOVIR GPO300) WITH PRIVATELY MANUFACTURED TENOFOVIR (VIREAD) USED ALONG WITH LAMIVUDINE AND EFAVIRENZ TO TREAT THAI HIV PATIENTS

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Abstract. The Thai Government Pharmaceutical Organization (GPO) has produced a nucleotide reverse transcriptase inhibitor, tenofovir disoproxil fumarate (Tenofovir GPO300). No clinical trial to date has compared plasma tenofovir concentrations, renal function, and treatment responses in HIV-infected patients who received Tenofovir GPO300 versus Viread (original tenofovir) as part of an antiretroviral regimen. We studied 129 antiretroviral treatment (ART)-naive HIV-1 infected patients who received an antiretroviral regimen of lamivudine, efavirenz and Tenofovir GPO300 (n=65) or Viread (n=64). We examined plasma tenofovir concentrations (12 hours after dosing), serum creatinine, estimated glomerular filtration rate (eGFR) using the Modification in Diet in Renal Disease (MDRD) study formula, fractional excretion of phosphate (FEphos), CD4 and plasma HIV-1 RNA levels at 12 weeks, and CD4 and plasma HIV-1 RNA levels at 24 weeks after initiating the drugs. At baseline, the mean±SD subject body weight was 54±10 kilograms and the mean±SD subject age was 37±8 years. At baseline, the median (IQR) CD4 count was 44 (18-120) cells/mm^3 and the median (IQR) HIV-1 RNA level was 5.8 log copies/ml. At baseline, the mean±SD eGFR was 134.8±43.6 ml/min/1.73 m^2. The baseline values for the two groups were not significantly different from each other (p>0.05). At 12 weeks, the mean±SD plasma tenofovir concentration was 106.9±41.5 ng/ml among the patients who received Tenofovir GPO300 and 100.7±49.4 ng/ml among those who received Viread (p=0.437). At week 12, there were no differences between those who rceived Tenofovir GPO300 and Vilead in mean serum creatinine (0.78 *vs* 0.81 mg/dl, p=0.283), mean eGFR (117.9 *vs* 109.1 ml/min/1.73 m^2, p=0.089), decline in eGFR from baseline (-21.8 *vs* -20.6 ml/min/1.73 m^2, p=0.860) or mean FEphos (11.4 *vs* 11.2, p=0.923). The median CD4 cell counts and number of patients with undetectable plasma HIV-1 RNA at week 24 were not significantly different (p>0.05) between those who took Tenofovir GPO300 and Viread. In summary, plasma tenofovir concentrations, changes in renal function, urinary phosphate excretion and treatment responses were comparable between HIV-infected patients who received Tenofovir GPO300 and Viread-containing non-nucleoside reverse transcriptase regimens.

Keywords: Tenofovir GPO300, tenofovir, HIV, Thailand

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INTRODUCTION

Tenofovir disoproxil fumarate (TDF) has been widely prescribed as a part of nucleoside reverse transcriptase inhibitor (NRTI) backbone for the treatment of HIV-1 infection in both resource-rich and resource-constrained settings (Gazzard *et al*, 2008). Current HIV treatment guidelines recommend TDF as a backbone drug for first-line HIV treatment. It may also be used for treatment of HIV-1 resistant to other nucleoside reverse transcriptase inhibitors (Department of Health and Human Services and the Henry J Kaiser Family Foundation, 2009), particularly in resource-limited settings where other new drug classes are still limited (WHO, 2013). Tenofovir has few side effects or toxicities. The most frequent adverse events reported in clinical trials are mild gastrointestinal effects, such as nausea, flatulence and diarrhea (Nelson *et al*, 2007). Severe renal toxicity, including renal dysfunction and tubular dysfunction, has been reported infrequently to date (Gallant *et al*, 2004; Jones *et al*, 2004; Nelson *et al*, 2007; Gallant *et al*, 2008; Smith *et al*, 2009).

TDF is patented under the brand name Viread. The Thai public health policy for antiretroviral therapy (ART) is to avoid death, disease progression, HIV transmission, and support the development of treatment programs that can reach as many patients as possible (Sungkanuparph *et al*, 2010). Thailand is among the few developing countries that has achieved nearly universal access to ART (Sungkanuparph *et al*, 2010). One of the responsibilities of the Thai Government Pharmaceutical Organization (GPO), is to increase access to ART and provide antiretroviral drugs for the national ART program. The Thai GPO began research on and development of ART drugs in 1992. Generic TDF (Tenofovir GPO300) is one of the ART in this program. However, clinical data regarding TDF drug levels, complications and treatment responses to Tenofovir GPO300 among HIV-infected patients are limited. Therefore, we conducted a prospective study to determine plasma TDF concentrations, renal complications and immunological and virological responses to Tenofovir GPO300-containing ART regimens among HIV-infected Thai patients with a baseline CD4 cell count <350 cells/mm^3.

MATERIALS AND METHODS

We conducted a prospective, open-label trial among 129 consecutive adult HIV-infected Thais to determine plasma TDF concentrations, renal function, urinary phosphate excretion, CD4 counts and HIV viral RNA levels at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. The first 64 patients received Viread and the next 65 patients received Tenofovir GPO300. The institutional ethics committee of Bamrasnaradura Infectious Diseases Institute approved the study. All participants gave written, informed consent prior to participation. Study enrollment was conducted between October 2009 and May 2011. Subjects were followed for 24 weeks after initiation of ART. A plasma TDF concentration were obtained after 12 weeks of ART. Renal function, urinary phosphate excretion, CD4 counts and HIV RNA levels were determined at 12 and 24 weeks after ART initiation. Adherence with the ART was determined by self reporting. Inclusion criteria included: 1) HIV-infected patients aged 18-60 years, 2) naïve to ART, 3) having a pre-ART CD4 cell count <350 cells/mm^3, 4) who were willing to participate in the study.

Exclusion criteria were: 1) having a serum creatinine level >2 times the upper limit of normal prior to initiation of ART and 2) having HIV genotypic resistance to any antiretroviral drug in the study. The primary objective of the study was to compare plasma TDF concentrations among HIV-infected patients who received either Tenofovir GPO300- or Viread- containing ART regimens. The secondary objectives were to compare renal complications and immunological and virological responses between subjects receiving the 2 different TDF ART regimens.

All the participants were started on a once daily antiretroviral regimen containing TDF 300 mg, lamivudine 300 mg and efavirenz 600 mg. Sixty-five patients received the TDF as GPO300 and 64 patients received the TDF as Viread. The patients had follow-up visits at 2 weeks, 6 weeks, 12 weeks and 24 weeks after initiating ART, when they were assessed clinically and blood samples were taken. All the patients were instructed to take their medications.

CD4 cell counts were measured by flow cytometry (TriTEST; Becton Dickinson BioSciences, San Jose, CA) analyzed with a FACScan flow cytometer (Becton Dickinson BioSciences, San Jose, CA). The plasma HIV-1 RNA viral load was evaluated by real-time PCR using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche Molecular Systems, Branchburg, NJ). TDF levels were obtained 12 hours after dosing using a validated high performance liquid chromatopraphy assay. This assay was developed at the Department of Clinical Pharmacology, University Medical Centre Nijmegen, The Netherlands. Serum creatinine, plasma phosphate, spot urine creatinine, and spot urine phosphate levels were obtained fasting. The fractional excretion of phosphate (FEphos) was calculated as: FEphos = (UPO4 x Pcreatinine x 100) ÷ (PPO4 x Ucreatinine), where UPO4 = urine phosphate, Pcreatinine = plasma creatinine, PPO4 = plasma phosphate and Ucreatinine = urine creatinine. The estimated glomerular filtration rate (eGFR) was calculated by the Modification in Diet in Renal Disease (MDRD) and Thai eGFR formula. The eGFR MDRD was calculated as: 186 x serum creatinine$^{-1.154}$ x age$^{-0.203}$ x (0.742 if female). The Thai eGFR formula was calculated as: 186 x serum creatinine$^{-1.154}$ x age$^{-0.203}$ x (0.742 if female) x 1.129. Genotypic resistance testing (TRUGENE HIV-1 Genotyping Assay; Visible Genetics, Toronto, Canada) was performed at week 0 prior to ART initiation.

Frequencies and medians, with interquartile ranges (IQR), were used to describe clinical variables and laboratory variables. A p <0.05 was considered statistically significant. Inter-patient variability in plasma TDF concentrations was expressed as a coefficient of variation (CV). The analyses were performed using the intention-to-treat principle. All analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL).

RESULTS

At baseline the mean±SD participant body weight was 54±10 kilograms and the mean±SD age was 37±8 years. The median (IQR) baseline CD4 count was 44 (18-120) cells/mm^3 and the median (IQR) plasma HIV-1 RNA level was 5.8 log copies/ml. At baseline, the mean±SD serum creatinine level was 0.72±0.18 mg/dl and the mean±SD eGFR was 134.8±43.6 ml/min/1.73 m^2. Most of the baseline characteristics were not different between the two TDF groups but baseline HIV RNA levels were significantly higher in the Viread group than the Tenofovir

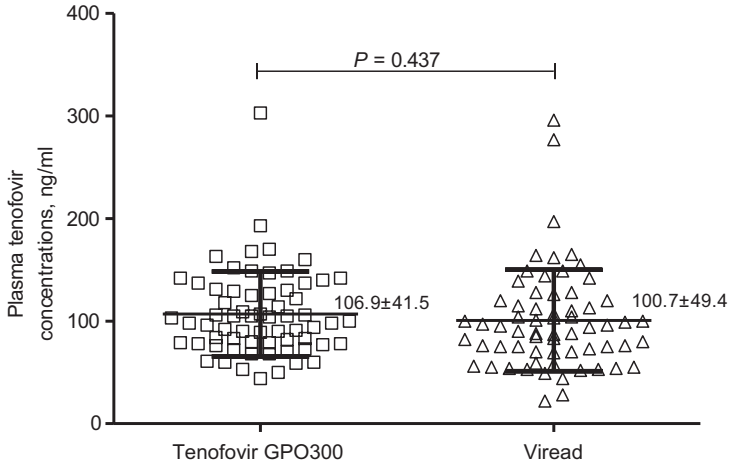

Fig 1–Scatter plot of plasma tenofovir concentrations at 12 weeks among HIV-infected patients who received Tenofovir GPO300 and Viread. Each dot represents one patient. The middle bars indicate the means; and the upper and lower bars represent the standard deviations of the means.

GPO300 group (p=0.008) (Table 1). Fig 1 shows the mean±SD plasma TDF concentrations in both groups (p=0.437). The inter-patient variability in plasma TDF concentrations was among those who received Tenofovir GPO300 was 38.8 and among those who received Viread was 46.6. Table 2 compares the secondary outcomes between the two groups. There were no differences in terms of mean FEphos, mean serum creatinine, mean eGFR, median immunological, and median viological responses between the two treatment groups (p >0.05). At 24 weeks, 2 patients and

Table 1
Comparison of clinical characteristics and laboratory parameters between HIV-infected patients who received Tenofovir GPO300 and Viread.

Parameters	Tenofovir GPO300 n = 65	Viread n = 64	p-value
Male sex	50 (77%)	49 (76%)	1.000
Age in years, mean±SD	36.6 ± 8.2	37.8 ± 8.9	0.429
Bodyweight in kgs, mean±SD	53.6 ± 10.9	54.9 ± 9.5	0.466
Serum BUN in mg/dl, mean±SD	9.5 ± 3.7	10.0 ± 4.2	0.482
Serum creatinine in mg/dl, mean±SD	0.71 ± 0.19	0.73 ± 0.17	0.490
eGFR MDRD in ml/min/1.73 m^2, mean±SD	139.7 ± 51.2	129.8 ± 34.0	0.197
Thai eGFR in ml/min/1.73 m^2, mean±SD	145.7 ± 53.5	135.2 ± 36.9	0.196
Serum alanine aminotransferase in U/l, mean±SD	39.3 ± 26.2	33.3 ± 20.6	0.157
CD4 count in cells/mm^3, mean±SD	70 ± 67	80 ± 76	0.427
CD4 cell count in %, mean±SD	8 ± 7	8 ± 7	0.622
Plasma HIV-1 RNA in copies/ml, median (IQR)	456,000 (189,500 - 959,000)	884,000 (359,250 - 2,350,000)	0.008
Log plasma HIV-1 RNA in log copies/ml, median (IQR)	5.7 (5.3 - 6.0)	6.0 (5.6-6.4)	0.007
Hepatitis B virus antigen positive	4 (6.1%)	3 (4.7%)	1.000
Hepatitis C antibody positive	11 (16.9%)	6 (9.3%)	0.298

Table 2
Comparison of the secondary outcomes between HIV-infected patients who received Tenofovir GPO300 and Viread.

Parameters	Tenofovir GPO300 $n = 65$	Viread $n = 64$	p-value
Mean plasma phosphorus level in mg/dl	11.4 ± 8.6	11.2 ± 9.7	0.923
Fractional excretion of phosphate	3.6 ± 0.6	3.7 ± 1.0	0.412
Serum BUN at week 12	10.7 ± 3.5	11.7 ± 3.9	0.142
Serum creatinine at week 12	0.78 ± 0.17	0.82 ± 0.20	0.283
Mean eGFR MDRD at week 12	117.9 ± 32.7	109.1 ± 24.6	0.089
Mean Thai eGFR at week 12	115.7 ± 33.5	108.8 ± 27.1	0.619
Mean decline in eGFR MDRD at week 12 from week 0, ml/min/1.73 m^2	-21.8 ± 44.4	-20.6 ± 32.2	0.860
Serum BUN at week 24	11.2 ± 7.5	10.8 ± 3.5	0.672
Serum creatinine at week 24	0.90 ± 0.42	0.87 ± 0.24	0.569
Mean eGFR MDRD at week 24	103.9 ± 35.7	102.3 ± 25.5	0.778
Mean Thai eGFR at week 24	125.7 ± 34.9	136.7 ± 13.54	0.204
Mean decline in eGFR MDRD at week 24 from baseline in ml/min/1.73 m^2	-35.8 ± 54.3	-27.4 ± 33.2	0.292
CD4 count at week 24 in cells/mm^3, mean±SD	205 ± 135	208 ± 125	0.916
CD4 cell count at week 24 in %, mean±SD	13 ± 7	13 ± 7	0.980
Number (%) of patients with viral load <40 copies/ml at week 24	31 of 62 (50)	32 of 63 (51)	1.000
Number (%) of patients with viral load <500 copies/ml at week 24	59 of 62 (95)	59 of 63 (94)	1.000

eGFR MDRD, Estimated glomerular filtration rate Modification in Diet in Renal Disease.

1 patient in the Tenofovir GPO300 group were lost to follow-up and died, respectively. The cause of death was acute pneumonitis. One patient in the Viread group was lost to follw-up.

DISCUSSION

The plasma TDF concentrations, changes in renal function, urinary phosphate excretion and treatment responses were not significantly different between participants who received Tenofovir GPO300 and participants who received Viread-containing ART regimens. Elevated intracellular and plasma TDF concentrations are associated with renal toxicity (Ray *et al*, 2006). A study among Caucasians found a high mean trough plasma TDF concentration of 182 ng/ml among patients with renal tubular dysfunctions and 106 ng/ml among patients without renal tubular dysfunction (Rodriguez-Novoa *et al*, 2010). Having a trough plasma tenofovir concentration greater than 160 ng/ml was associated with a 5 times greater risk of developing renal dysfunction (Rodriguez-Novoa *et al*, 2010). A number of previous studies found a high plasma TDF concentration was associated with renal tubular dysfunction (Barditch-Crovo *et al*, 2001; Peyriere *et al*,

2004; Rodriguez-Novoa *et al*, 2010). In the present study, the mean plasma TDF concentrations in both study groups were approximately 100 ng/ml and none of the patients developed renal dysfunction, although there was a slight decline in eGFR. Several studies have found TDF-related adverse renal events *in vivo*, but this drug was found to be safe and well-tolerated in premarketing clinical trials (Gallant *et al*, 2004; Manosuthi *et al*, 2010; 2011). In a recent meta-analysis of 17 studies evaluating renal safety of TDF in HIV-infected paients, a reduction in renal function and an increase risk of renal failure were reported (Cooper *et al*, 2010). The cause of the proximal renal tubular dysfunction associated with TDF appears to be multi-factorial (Fernandez-Fernandez *et al*, 2011). However, the risk appear to outweigh the benefit of TDF.

The clinical presentations of TDF-related renal toxicity are: 1) proximal renal tubular impairment with preserved renal function and 2) proximal renal tubular impairment associated with impaired renal function (Fernandez-Fernandez *et al*, 2011). Impaired renal function may present as acute renal injury, chronic kidney disease or a dereased glomerular filtration rate (Fernandez-Fernandez *et al*, 2011). A urinary phosphate excretion rate above 100 mg per day or a FEphos above five percent is indicative of renal phosphate wasting. In the present study, approximately 80% of patients had a FEphos greater than this threshold indicating abnormal renal phosphate wasting, although only a small proportion of patients had a decreased glomerular filtration rate. Proximal renal tubular dysfunction may preceed a decline in renal function. A recent meta-analysis found a significantly greater loss of renal function among HIV-infected patients receiving TDF compared to the control group (Cooper *et al*, 2010). In our study, no clinically significant hypophosphatemia or Fanconi syndrome was seen in patients with excessive urinary phosphate loss. Severe hypophosphatemia is defined as a fasting phosphate level of less than 1.0 mg/dl after 12 hours (Bagnis *et al*, 2009). Detection of mild renal proximal tubular dysfunction is relatively difficult because urinary phosphate loss is usually compensated by bone loss to maintain a stable plasma phosphate level. This may explain why the mean serum phosphate level was normal in this study. The immunological and virological responses in our study were not different between the two treatment groups although the follow-up period was relatively short. Long-term studies to confirm our results are needed.

There were some limitations in this study. Extensive pharmacokinetics regarding the studied drug were not evaluated in our study. Other causes for urinary phosphate loss were not determined, such as vitamin D deficiency, hyperparathyroidism or other drug-induced tubulopathy. Several studies have found vitamin D deficiency to be relatively common among HIV-infected patients, especially among patients with inadequate exposure to sunshine (Adeyemi *et al,* 2011; Cervero *et al*, 2012). Inadequate exposure to sunshine is uncommon in tropical countries. In our study, no other tubulopathic drugs were prescribed, such as cidofovir, adefovir, aminoglycosides or cisplatin. This means TDF is the most likely cause of tubular dysfunction among our studied patients. Only a single plasma TDF level was obtained in our study. Intra-patient variation in TDF clearance has been reported (Gagnieu *et al*, 2008). The study design was not a randomized trial. The baseline median plasma HIV RNA level

was higher among patients who received Viread. However, this factor did not appear to influence the primary and secondary outcomes of our study. The sample size was relatively small. Thus, a larger study is needed to confirm our findings.

The introduction of generic antiretroviral drugs by the Thai government has significantly increased access to HIV treatment in Thailand. This study provides evidence regarding the safety, treatment response and quality of the generic TDF, Tenofovir GPO300.

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