AIDS Diarrhea and Antiretroviral Drug Concentrations: A Matched-Pair Cohort Study in Port au Prince, Haiti

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Abstract. Diarrhea in patients with acquired immunodeficiency syndrome (AIDS) may cause malabsorption of medications and failure of antiretroviral therapy (ART). We prospectively evaluated human immunodeficiency virus-1 (HIV-1)-infected patients with and without chronic diarrhea initiating ART in Haiti. We report mean plasma antiretroviral concentrations at 2 and 4 weeks. We measured plasma HIV-1 RNA levels at four points. Fifty-two HIV-1-infected patients (26 matched pairs) were enrolled. No differences in antiretroviral concentrations were detected. At week 24, 18/25 (72%) cases and 16/24 (68%) controls had undetectable plasma HIV-1 RNA levels ($P = 0.69$). Patients with plasma HIV-1 RNA levels > 50 copies/mL at week 24 had lower early efavirenz concentrations than patients with undetectable HIV-1 RNA (2,621 ng/mL versus 5,278 ng/mL; $P = 0.02$). Diarrhea at ART initiation does not influence plasma concentrations of the medications evaluated. Virologic outcome at Week 24 does correlate with efavirenz concentrations early in therapy but not with the presence of chronic diarrhea.

BACKGROUND

Chronic diarrhea is a common problem in patients with acquired immunodeficiency syndrome (AIDS) in Haiti and other resource-limited settings.^{1–5} Observational studies suggest that chronic diarrhea is associated with increased mortality in patients with AIDS, even after initiation of antiretroviral therapy (ART).^{6,7} Some have reported poor absorption of antimicrobial medications in patients with diarrhea and suggested that the increased mortality in patients with AIDS diarrhea may be related to malabsorption of antiretroviral drugs.^{8–13} Low plasma concentrations of some antiretroviral drugs predict treatment failure.^{14–17}

Therefore, we performed a prospective study to evaluate whether human immunodeficiency virus (HIV)-infected patients with chronic diarrhea at the time of ART initiation have lower plasma antiretroviral drug concentrations and higher rates of virologic failure compared with patients without chronic diarrhea.

METHODS

Study setting. The study was conducted at the Groupe Haitien d'Etude du Sarcome de Kaposi et des Infections Opportunistes (GHESKIO) Center in Port-au-Prince, Haiti. Patients with AIDS at GHESKIO receive treatment according to guidelines published by the World Health Organization (WHO),¹⁸ and protocols and outcomes have been described previously.^{19,20}

Study design. This was a matched-pair cohort study. We recruited HIV-1-infected patients who were initiating ART at GHESKIO who reported 3 weeks of diarrhea and controls also initiating therapy matched for age, sex, and CD4 count. All participants were initiated on antiretroviral medica-

tions and followed for 24 weeks. Plasma drug concentrations were measured at 2 and 4 weeks. Plasma HIV-1 RNA levels were measured at baseline, 2 weeks, 4 weeks, and at 24 weeks. We compared drug concentrations and HIV-1 RNA levels between patients with diarrhea and controls.

Study population. Entry criteria included: HIV-1 infected; age ≥ 18 years; satisfy WHO criteria for initiating ART; a hemoglobin > 7.5 mg/dL; creatinine $\leq 1.5 \times$ upper limit of normal (ULN), aspartate (AST) aminotransferase (previously SGOT), alanine aminotransferase (ALT) (previously SGPT) $\leq 3 \times$ ULN, and total bilirubin $\leq 2.5 \times$ ULN.

Exclusion criteria included: prior antiretroviral exposure and requirement for medications known or predicted to interact with antiretrovirals.

GHESKIO clinicians asked patients initiating ART about the presence or absence of diarrhea. A patient was offered enrollment as a case (diarrhea) if he/she reported at least 3 weeks of loose stools $> 3 \times$ per day. Once a case (diarrhea) patient was recruited, the research team worked with the clinicians in the ART clinic to identify and recruit a control matched on age within 5 years of the case; CD4 count in the same range (< 50 ; 51–100; 101–200; > 200); and sex.

All participants initiated therapy with efavirenz (EFV, 600 mg every 24 hours in the evening) plus fixed-dose zidovudine/lamivudine (ZDV/3TC, 300/100 mg every 12 hours). Directly observed therapy was used to assure adherence for the first 4 weeks of therapy. Study personnel visited the participants' homes each morning to observe the ingestion of the morning dose. A family member or friend, designated by the participant, observed and documented the evening doses. All observed doses were recorded in a pictorial medication diary.

All participants were followed by study personnel for 24 weeks after the initiation of therapy with study visits at 2, 4, and 24 weeks.

Laboratory assays. Screening tests for kidney and liver function were performed: AST (SGOT), ALT (SGPT), and creatinine (VITROS; Ortho Clinical Diagnostics, Raritan, NJ). A complete blood count was done at baseline and at

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each study visit (Cell-Dyn; Abbott Laboratories, Abbott Park, IL). A CD4 T cell count was measured at baseline and at 24 weeks (Becton, Dickinson, Franklin Lakes, NJ). The plasma HIV-1 RNA level was performed at baseline, 2 weeks, 4 weeks, and 24 weeks (NASBA Easy Q; bioMérieux, Lyons, France).

The d-xylose carbohydrate absorption test was performed at baseline and at 2 and 24 weeks. Participants fasted for 8 hours before coming to the clinic. To perform the test, study personnel provided each participant with 25 grams of d-Xylose (Xylo PFAN, Savage Laboratories, Melville, NY) dissolved in 250 mL of water. The participant was then asked to drink an additional 250 mL of water. Urine was collected in a large plastic container over the next 5 hours. A 25 mL aliquot was preserved in sodium fluoride (NaF), and the total volume of the sample was recorded.

A stool sample was collected at enrollment for each patient. Routine examinations performed by trained technologists at GHESKIO included: guaiac testing, methylene blue staining on a wet mount for white blood cells, and a microscopic exam for ova and parasites. Coccidial oocysts were identified with modified Kinyoun acid-fast staining. Aliquots of unpreserved stool were frozen for batch testing by polymerase chain reaction for *Cryptosporidium* sp., enteroaggregative *Escherichia coli*, *Campylobacter jejuni*, *Clostridium difficile*, and *Enterocytozoon bineusi*.^{21–26} Quantitative lactoferrin enzyme-linked immunosorbent assay (IBD-Scan; Techlabs, Blacksburg, VA) was also performed on frozen stool samples. Antimicrobial susceptibility testing was performed by disk diffusion.

Blood plasma samples were obtained from patients when they came to the GHESKIO clinic for their morning dose of ART on the day of the 2-week and 4-week study visits. Plasma samples for drug assays were obtained before the morning dose of ZDV/3TC. Time since the last doses of efavirenz, ZDV, and 3TC were documented. Plasma was separated by centrifugation at 4°C and stored at –70°C. Efavirenz, ZDV, and 3TC were quantified using two validated methods in the UNC Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core.^{27,28} The dynamic range for efavirenz was 25 to 10,000 ng/mL, intra- and interday precision of 4.8–5.5%, and accuracy of 100.4–101.7%. The dynamic range for ZDV and 3TC was 10–10,000 ng/mL, with intra- and interday precision of < 7%, and accuracy of ≥ 90% for all concentrations. Samples below level of quantitation (BLQ) were considered to be 50% of the lowest level detectable of the assay. Samples below level of detection (BLD) were considered to be “0.”

Statistical analysis. Data were analyzed using SPSS version 17.0 (Chicago, IL). The primary analysis was to compare antiretroviral drug concentrations and plasma HIV-1 RNA levels between patients with diarrhea and controls. For each participant a single plasma drug concentration value derived and based on the geometric mean of Week 2 and Week 4 values for zidovudine, lamivudine, and efavirenz was used for statistical analyses. In one individual without Week 2 data and one individual without Week 4 data, the Week 4 and Week 2 values were used to represent geometric mean, respectively. The geometric mean concentrations for each drug were then used to determine medians, interquartile ranges (IQRs), and correlations among groups. The median values were compared between groups using Wilcoxon rank sum test for paired variables and Mann-Whitney test for unpaired variables.

The HIV-1 RNA levels at baseline and Week 2 were log₁₀ transformed, and we reported the decrease from baseline at 2 weeks. The median values and IQRs were reported for each group, and medians were compared between groups using the Wilcoxon rank sum test. At Week 24, we reported the percent of participants with plasma HIV-1 RNA level < 50 copies/mm³ and compared groups using the χ^2 squared test. To correct for multiplicity of primary endpoints, we set the significance level at a *P* value of 0.01.

Baseline characteristics and clinical outcomes between index and control participants were compared using a χ^2 test for categorical variables and the Mann-Whitney test for continuous variables. Significance was pre-assigned as *P* < 0.05.

Ethical review. Ethical review boards at GHESKIO, Weill Cornell Medical College, and the University of Virginia approved the study.

RESULTS

Baseline characteristics of study participants at initiation of antiretroviral therapy. Twenty-six HIV-1 infected patients with active diarrhea were enrolled. A matched control was enrolled for each case. Of note, one matched-control patient died 9 days after enrollment because of a cerebral infection. An additional control was subsequently recruited as a replacement. The data from the deceased patient is not included in this analysis.

The baseline clinical and demographic characteristics of the 26 patients with and 26 without chronic diarrhea are presented in Table 1. The low median CD4 count and high HIV RNA level show advanced HIV disease and immune compromise in all patients. Women with diarrhea had significantly lower body mass index and hemoglobin levels compared with women without diarrhea. The patients with diarrhea had a significantly higher mean quantitative lactoferrin in their stool samples compared with those without diarrhea (37.83 µg/mL versus 14.71 µg/mL; *P* = 0.001). Lactoferrin is a marker of gastrointestinal inflammation. There was a trend toward lower urine d-xylose excretion in patients with diarrhea compared with those without diarrhea. The d-xylose excretion is a measure of gastrointestinal carbohydrate absorptive capacity.

At enrollment, 47/52 (88%) participants provided a stool sample. Stool analysis revealed that the case and control populations had similar rates of enteric pathogen identification (Table 1). Enteroaggregative *E. coli* (EAEC) was the most commonly identified pathogen (29/46; 63%). 5/6 (83%) of EAEC isolates tested were resistant to trimethoprim-sulfamethoxazole.

All patients in the study were initiated on 3-drug ART. For the patients with chronic diarrhea, the median duration of diarrhea following initiation of antiretroviral therapy was 19 days (IQR 14–27 days).

Plasma antiretroviral drug levels and HIV-1 RNA levels. Plasma samples for drug concentrations of both ZDV/3TC and efavirenz were obtained 14 (IQR 13–15) hours and 13 (IQR 13–14) hours after the previous doses at 2 and 4 weeks, respectively. Mean plasma antiretroviral drug concentrations did not differ between patients with diarrhea and controls at Week 2 or Week 4; nor did the geometric mean values for each antiretroviral drug differ, Table 2. The geometric mean zidovudine concentration was actually higher for patients with diarrhea (121.86 ng/mL versus 32.36 ng/mL, *P* = 0.18). There

TABLE 1
Baseline characteristics of index and control patients*

Variable		Patients with chronic diarrhea (N = 26)	Patients without chronic diarrhea (N = 26)	P value
Matched characteristics for each pair	Age(years) (median [IQR])	38 [32–44]	37 [34–44]	0.832
	% female	54	54	1.000
	Baseline CD4 count (cells/mm ³) (median [IQR])	59 [21–148]	64 [24–151]	0.967
	Baseline viral load (copies/mm ³) (median [IQR])	250,000 [125,000–845,000]	145,000 [57,000–277,000]	0.339
	% living on < \$1/day	55	54	0.827
	% living with partner	50	42	0.578
	% Completing primary education or above	62	50	0.602
	% who were on TMP-SMX prophylaxis	96	92	1.000
	BMI (kg/m ²): females (kg/m ²) (median [IQR])	20.3 [17.2–21.5]	23.1 [21.3–25.1]	0.004
	BMI(kg/m ²): males (median [IQR])	20.2 [17.5–24.8]	20.5 [19.1–23.6]	0.934
	Hemoglobin (gm/dL): females (median [IQR])	9.4 [8.7–10.4]	10.6 [9.9–11.4]	0.014
	Hemoglobin (gm/dL): males (median [IQR])	10.5 [9.3–12.9]	11.7 [10.3–12.6]	0.492
	D-xylose excretion (%; normal > 16%): (median [IQR])	14.3 [5.4–24.6]	18.4 [13.4–29.2]	0.275
Stool pathogens identified by PCR analysis	Fecal lactoferrin (mcg/mL)	37.8	14.3	0.001
	<i>Cryptosporidium</i> sp.	7/23 (30%)	10/23 (44%)	0.359
	Enterocoagulative <i>E. coli</i>	16/23 (70%)	13/23 (57%)	0.359
	<i>E. bieneusi</i>	3/23 (13%)	4/23 (17%)	0.681
	<i>C. jejuni</i>	3/23 (13%)	2/23 (9%)	0.636
	<i>C. difficile</i>	2/23 (9%)	1/23 (4%)	0.550
	2 or more pathogens	10/23 (44%)	10/23 (44%)	1.000
	No pathogens	4/23 (17%)	5/23 (22%)	0.710

* IQR = interquartile range; TMP-SMX = trimethoprim-sulfamethoxazole; BMI = body mass index; PCR = polymerase chain reaction.

was no association between plasma drug concentrations and particular enteric pathogens, quantitative lactoferrin levels, or d-xylose excretion.

There was no important difference in plasma HIV-1 RNA levels between cases with diarrhea and controls at Week 2, Week 4, or Week 24. At Weeks 2 and 4, the patients with diarrhea tended to have a greater reduction in viral load than controls. At 2 weeks, the patients with diarrhea had a median log-transformed reduction in viral load of 5.39; patients without diarrhea had a log-transformed reduction of 5.14 ($P = 0.07$). At 4 weeks, a similar pattern in viral load reduction remained (5.40 versus 5.17; $P = 0.07$). At Week 24, 18/25 (72%) patients with diarrhea and 16/24 (68%) patients without diarrhea had achieved an undetectable viral load ($P = 0.69$).

Of note, the patients who achieved an undetectable viral load at Week 24, regardless of the presence or absence of diarrhea at baseline, had higher plasma drug concentrations for all three antiretrovirals at Weeks 2 and 4 and by geometric mean. The difference in EFV levels was significant (Table 3 and Figure 1).

Other measures of treatment response. At 24 weeks, both groups had good immunologic responses. Patients with diarrhea had a median increase of 119 cells/mm³ (IQR: 50–174 cells/mm³), and patients without diarrhea had a median increase of 120 cells/mm³ (IQR: 77–158 cells/mm³).

Patients with diarrhea tended to gain more weight than patients without diarrhea. At Week 4, the patients with diar-

rhea had significantly greater weight gain compared with those without diarrhea (3% versus 1% of total body weight; $P = 0.02$). By 24 weeks, the group with diarrhea had still gained more weight (13.5% versus 9.0% of total body weight; $P = 0.243$). By 24 weeks, the urine d-xylose excretion had normalized in both groups with a mean of 30.7% in the patients with diarrhea and a mean of 31.7% in the controls.

DISCUSSION

This study demonstrates that AIDS diarrhea at the initiation of ART does not significantly affect drug concentrations of AZT, 3TC, or EFV in this profoundly immune-suppressed population with adequate renal and hepatic function. After initiation of ART, plasma HIV-1 RNA levels and other measures of treatment response were similar between patients with AIDS diarrhea and matched controls.

Our study prospectively examined plasma antiretroviral drug concentrations, plasma HIV-1-RNA levels, and clinical outcomes relative to the presence or absence of chronic diarrhea. The clinical response to ART by patients with diarrhea was at least as robust as matched controls without diarrhea. In fact, patients with diarrhea had a trend toward greater reduction in viral load measured at Week 2 and Week 4 than those without diarrhea. They had an equally good rise in CD4 count, achievement of undetectable HIV viral load at 24 weeks, and gain of weight.

TABLE 2
Antiretroviral drug concentrations and HIV-1 RNA levels in patients with and without diarrhea*

Measure	Patients with chronic diarrhea (N = 26)	Patients without chronic diarrhea (N = 26)	P value
Plasma zidovudine concentration (ng/mL) [IQR]	23.04 [BLD–74.36]	BLD [BLD–35.83]	0.07
Plasma lamivudine concentration (ng/mL) [IQR]	114.12 [79.54–175.51]	146.90 [78.72–194.57]	0.38
Efavirenz concentration (ng/mL) [IQR]	3066.32 [2361.55–6533.37]	3294.32 [1734.40–7069.68]	0.87
2-week change in log ₁₀ HIV-1 RNA level [IQR]	5.39 [5.09–5.93]	5.14 [4.7–5.43]	0.07
Proportion of participants with plasma HIV-1 RNA < 50 copies/mm ³ at 24 weeks	18/25 (72%)	16/24 (68%)	0.69

* BLD = below level of detection; IQR = interquartile range; HIV-1 = human immunodeficiency virus-1.

TABLE 3
Trough antiretroviral concentrations relative to achievement of non-detectable viral load at Week 24*

	Antiretroviral	Median values for patients with undetectable viral load at Week 24 (ng/mL) [IQR]	Median values for patients with detectable viral load at Week 24 (ng/mL) [IQR]	P value
Week 2	ZDV	20.20 [10.00–51.29]	14.50 [0.00–29.40]	0.25
	3TC	136.20 [77.88–200.45]	95.10 [47.20–170.60]	0.33
	EFV	3709.61 [2744.90–7750.10]	2974.70 [1770.30–3421.50]	0.03
Week 4	ZDV	31.40 [0.00–85.22]	10.50 [0.00–24.88]	0.11
	3TC	132.50 [94.10–216.73]	89.95 [67.85–142.30]	0.07
	EFV	3437.35 [2210.98–6510.33]	2046.00 [1461.90–3270.23]	0.02
Geometric mean	ZDV	28.00 [0.00–64.79]	0.00 [0.00–25.05]	0.06
	3TC	144.53 [87.05–207.26]	95.10 [55.29–160.85]	0.08
	EFV	3448.01 [2661.02–7487.07]	2307.41 [1336.04–3414.90]	0.01

* IQR = interquartile range; ZDV = zidovudine; 3TC = lamivudine; EFV = efavirenz.

A previous study showing a correlation between AIDS diarrhea and low antiretroviral drug concentrations examined patients who presented with diarrhea after initiation of ART.¹⁰ In this case, the occurrence of diarrhea during ART may have been an effect of poor adherence and treatment failure, rather than the cause. Other studies suggesting that AIDS diarrhea is in itself related to poor treatment outcome may not have controlled for HIV disease stage and severe immune suppression. As demonstrated in our study, chronic diarrhea is a clinical marker for advanced HIV disease; the median CD4 count of our patients with AIDS diarrhea was only 59 cells/mm³.⁶

Virologic outcome in our HIV-1 infected patients in Haiti correlated with plasma efavirenz concentrations early in therapy. Of note, patients in the current study received directly observed therapy and therefore differences in adherence do not explain the variation in efavirenz exposure. Rather, it appears that an individual's efavirenz exposure effects response to ART. The current study shows that low efavirenz exposures are not caused by diarrhea. Our previous analysis suggests that low efavirenz concentrations in patients in Haiti may be related to genetic polymorphisms in the cytochrome P450 (CYP) 2B6 and thus differences in metabolic rate.²⁹

Enterotoxigenic *E. coli* was common in our HIV-infected patients, which is consistent with previous reports.^{26,30,31} As documented in our patients, EAEC is frequently resistant to widely available antimicrobials.^{32,33} Its high prevalence and reports that infection may negatively impact nutrition suggest

that this pathogen and strategies for its management deserve further investigation.^{34,35}

In conclusion, patients with AIDS diarrhea have advanced immune suppression. However, they respond well to ART with no significant differences in drug exposure for ZDV, 3TC, and EFV. On the basis of these findings, we do not recommend delay in ART until cessation of diarrhea or a dosage adjustment of ZDV, 3TC, and EFV for patients with AIDS diarrhea. Rather, we encourage prompt initiation of ART at standard dosing for patients with AIDS diarrhea.

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FIGURE 1. Comparison of efavirenz (EFV) concentrations between individuals with detectable and undetectable viral loads at 24 weeks.

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