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Chronic ethanol use in alcoholic beverages by HIV-infected patients affects the therapeutic window of stavudine, lamivudine and nevirapine during the 9-month follow-up period: using chronic alcohol-use biomarkers

Abstract

Background: Chronic ethanol use is a global problem including among HIV-infected patients on stavudine/lamivudine/nevirapine (d4T/3TC/NVP) regimen. The study determined the effect of chronic ethanol use on the therapeutic window of d4T, 3TC and NVP in HIV-infected patients using alcohol-use biomarkers to screen patients for chronic ethanol use.

Methods: A case-control study using repeated measures design with serial measurements was used to quantify drugs in plasma. The WHO alcohol use disorder identification test (AUDIT) tool was initially used to screen patients for chronic alcohol use, and then they were further sorted using alcohol-use bioamarkers (γ-glutamyl transferase ≥55.0 IU; mean corpuscular volume, ≥96 fl, aspartate amino transferase/alanine aminotransferase ratio ≥2.0 value). A total of 41 patients (26 in the alcohol group and 15 in the control group) were followed up for 9 months with blood sampling done at 3-month intervals. Plasma drug concentrations were quantified using a Shimadzu Class-VP™ HPLC data system version 6.1. Data was analyzed using SAS 2003 version 9.1 statistical package with repeated measures fixed model. Means were compared using Student's t-test.

Results: The mean steady-state plasma drug concentrations of d4T and 3TC in the alcohol group were lower than that in the control group during the 9-month period of follow-up. For 3TC, there was a statistical difference in the mean steady-state plasma drug concentrations between the alcohol group and the control group ($p \leq 0.05$) in the 6- and 9-month period of follow-up. For NVP, in both groups they were within the reference ranges, although the drug plasma concentrations were higher in the alcohol group compared to the control group and were statistically significant ($p<0.05$) in 0, 3 and 6 months of follow-up.

Conclusions: Chronic ethanol use by HIV-infected patients reduced the therapeutic steady-state plasma drug concentrations of d4T and 3TC and increased the NVP drug concentrations in the HIV-infected patients.

Keywords: alcohol-use biomarkers; chronic ethanol use; HIV; lamivudine (3TC); nevirapine (NVP); stavudine (d4T); steady-state plasma drug concentrations; therapeutic window.

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Introduction

Chronic alcohol consumption is a serious problem globally. Ethanol is a major component in alcoholic beverages like wine, spirits, liquors, beers and traditional brew (homemade brew) [1–4]. Alcohol consumption in Uganda is a problem including among the HIV-infected patients on antiretroviral drug regimen like stavudine/lamivudine/nevirapine (d4T/3TC/NVP), and Uganda is ranked among the top alcohol consumers worldwide and in the African region [3, 5–10]. The high and sustained alcohol levels and its metabolites in the body can interact

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pharmacokinetically and pharmacodynamically with a number of medications the patients were taking such as d4T/3TC/NVP drug regimen. Ethanol is metabolized by alcohol dehydrogenase (ADH) enzyme isoforms (ADH1A, ADH1B, ADH1C, ADH4, ADH5, ADH6 and ADH7) to produce acetaldehyde and reactive oxygen species (ROS), and aldehyde dehydrogenase (ALDH) enzyme isoforms (ALDH1 and ALDH2) that metabolize acetaldehyde to produce energy, carbon dioxide and water. These enzymes are highly expressed and found mainly in the gut wall in males and in the liver in females [11–13]. Other ethanol-metabolizing enzymes are the hepatic microsomal ethanol-oxidizing systems (MEOS) including cytochrome 2E1 (CYP2E1), CYP1A2 and CYP3A4 [11–13], with CYP2E1 as the main hepatic alcohol metabolizer in the liver and catalase in the peroxosomes to produce ROS (Figures 1 and 2) [11–15]. The products of alcohol metabolism generate a number of potentially harmful by-products such as acetaldehyde, acetate, ROS such as hydroxyl radicals, superoxide anion and hydroxyl radicals and fatty acid ethyl esters. The accumulation of ethanol metabolites damages the liver, leading to raised serum enzymes, and also affects hemopoiesis [16, 17]. These also affect the serum enzyme levels and mean corpuscular volume (MCV), and hence, they are used as chronic alcohol-use biomarkers [18–21]. Biomarkers are cellular or molecular indicators of exposure, disease or susceptibility to disease, and in this case, the ethanol exposure. Other chronic alcohol-use biomarkers include the elevation of the carbohydrate-deficient transferrin, sialic acid in serum, ethyl glucuronide, ethyl sulfate, phosphatidyl ethanol, liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) and MCV [18, 21, 22]. However, the routinely used chronic alcohol-use biomarkers include GGT, MCV and AST/ALT ratio if above 2.0 [18, 21, 22]. The elevation of GGT values above 55.0 UI, MCV values above 96 fl and AST/ALT ratio above 2.0 are indicators of chronic alcohol use. The alcohol-use disorders identification test (AUDIT) tool is a noninvasive method and is commonly used to screen patients for chronic alcohol use [18].

The accumulation of ethanol metabolites in the body and the HIV virus can also damage the liver and the kidney of the patients, thus affecting the elimination of the administered drugs like d4T, 3TC and NVP from the body and this affects the therapeutic steady-state plasma drug concentrations that are available to suppress the HIV virus in the patients. d4T is a nucleoside reverse transcriptase inhibitor (NRTI) with a reported therapeutic steady-state plasma drug concentration (therapeutic window) range of 0.09–0.68 μg/mL (Table 1), and about 40%–50% of the administered dose is reported to be excreted as free drug in urine in unchanged form by the kidneys [26–32]. The excretion may be enhanced by the diuretic effect of ethanol [33, 34]. 3TC is also an NRTI with therapeutic steady-state plasma drug concentration range of 0.2–1.42 μg/mL (Table 1) [26–30, 32], and like d4T, about 70%–80% of the administered dose is excreted as unchanged drug or free drug in urine by the drug transporter involving active cationic secretion via glomerular filtration and tubular secretion [26–30, 32]. NVP is a non-NRTI (NNRTI) with a potent and selective inhibitor of the replication of a wide variety of HIV viral strains [26–30, 32, 35, 36]. NVP has a reported therapeutic steady-state plasma drug concentration range of 5.3–11.7 μg/mL (Table 1). More than 80% of the NVP dose administered is biotransformed by cytochrome P450 (CYP450) oxidation mainly by CYP3A, CYP2B6 and CYP2E1 that can also be autoinduced, into hydroxylated metabolites that are mainly excreted in urine as glucuronides (Figure 3) [26–30, 32, 37].

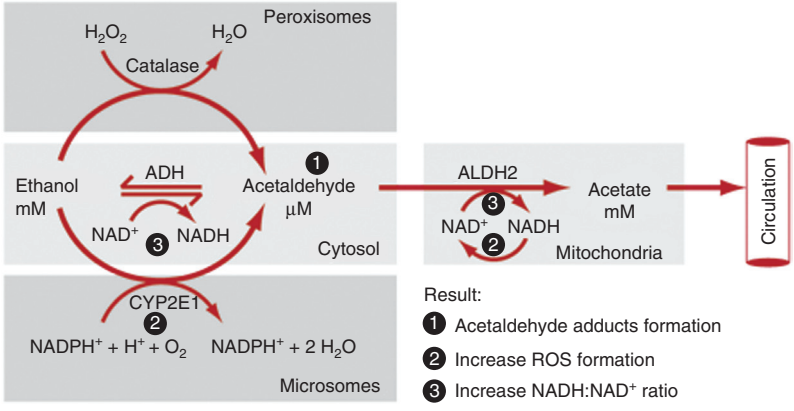


Figure 1 Oxidative pathways of ethanol metabolism (adopted from Zakhari [14]).
ADH, alcohol dehydrogenase; CYP2E1, cytochrome P450 2E1; NAD^+, nicotinamide adenine dinucleotide, reduced form of NADH; ALDH2, aldehyde dehydrogenase 2.

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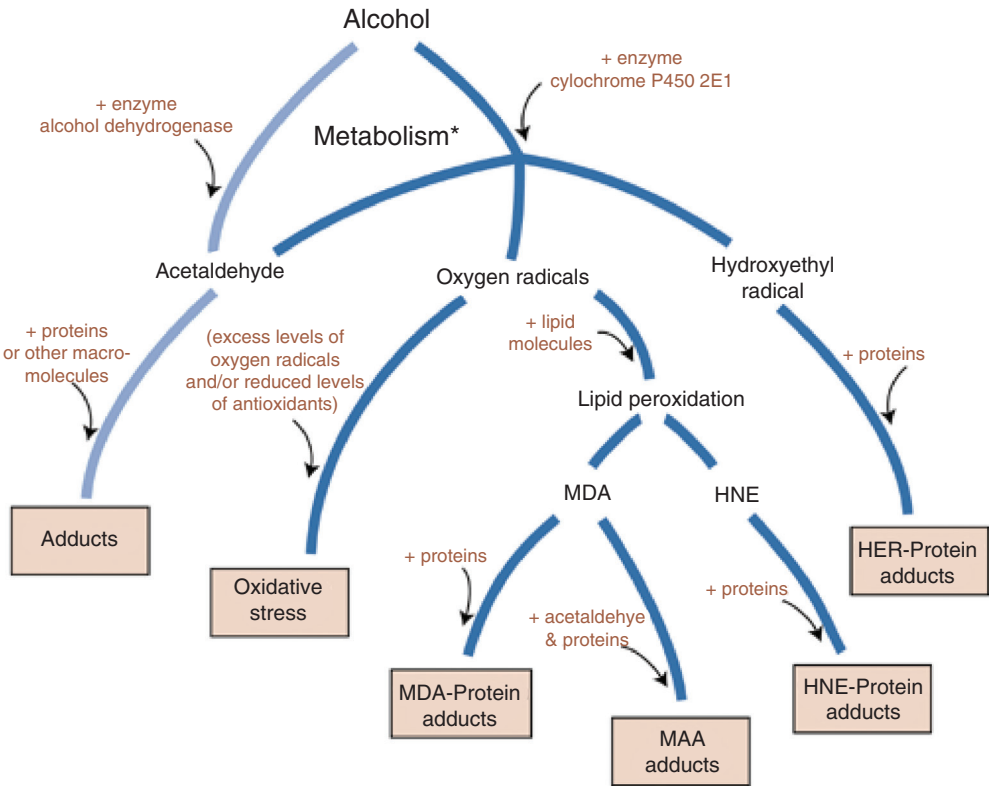


Figure 2 Potentially toxic products from ethanol metabolism (adopted from Tuma and Casey [15]).
MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; HER. hydroxyethyl radical; MAA, MDA-acetaldehyde-protein adducts.

Table 1 Therapeutic steady-state concentrations of d4T, 3TC and NVP [16, 17, 23–25].

Drug	Therapeutic window	
	Peak plasma concentration, µg/mL	Trough concentration, µg/mL
Stavudine	0.68	0.09
Lamivudine	1.42	0.2
Nevirapine	11.7	5.3

Also during species evolution, living organisms protect themselves from foreign substances (xenobiotics) such as drugs, poisons, toxins and nutrients by use of nuclear receptors such as aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR) that act as sensors (xenosensors) of the chemical substances and the activation of the xenobiotic metabolizing and transporter systems [38, 39]. The nuclear receptors detect xenobiotics such as drugs in the cells and coordinate the expression of genes encoding the most appropriate series of xenobiotic-metabolizing and transporter systems to inactivate and/or eliminate these substances from the body. The xenobiotic-metabolizing and transporter systems include CYP450, conjugation enzymes and drug transporters [38–40]. The nuclear receptors and transcription factors coordinate expression of many genes, and regulation of their expression determines cellular response to various endo- and exogenous factors. CAR and PXR are the two very important nuclear receptors, which regulate gene transcription of enzymes and membrane transporters in response to exo- and endogenous compounds, forming a line cellular defense against drugs and other toxins [38–40]. CAR and PXR regulate many overlapping sets of xenobiotic-metabolizing genes of CYP450 enzyme systems of phase I and phase II and the drug transporters. The nuclear receptor AhR activates CYP1A1 and CYP1A2, PXR and CAR activate CYP2B6, CYP2C9, CYP2C19, CYP3A4/5, and in addition, PXR activates CYP2C8 and CYP3A7 (expressed in fetal liver), whereas CAR also activates CYP2A6 [38–40]. The drug nevirapine has been reported to activate the PXR and CAR nuclear receptors following its exposure to body cells and then activates the metabolizing enzymes. After oral administration, NVP is readily absorbed (>90%) and freely partitions to all tissues, including the brain, due to its low level of protein binding. Hepatic metabolism of NVP is mainly through induction of CYP

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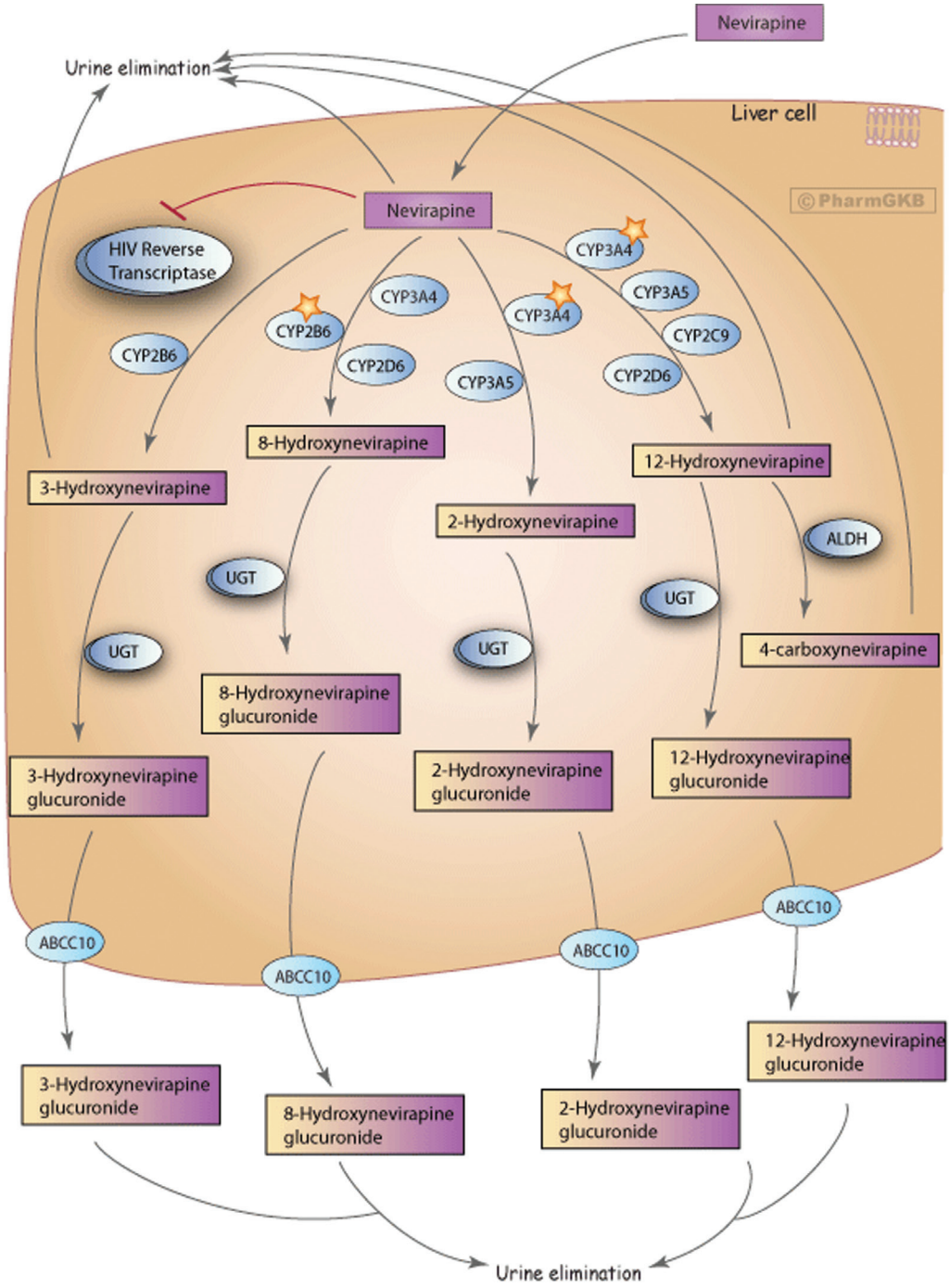


Figure 3 Nevirapine metabolic pathway and its elimination from the body (adopted from Dhoro and Sangkuhl; PharmGBK, 2012, with permission by PharmGKB and Stanford University) [37].

enzymes 3A4 and 2B6, leading to formation of 2-, 3-, 8-, and 12-hydroxynevirapine followed by glucuronidation of these hydroxyl metabolites by uridine 5′-diphospho-glucuronosyltransferase (UGT) enzymes (Figure 3) [35]. Other CYP enzymes, CYP3A5, CYP2C9 and CYP2D6, play a minor role. However, nevirapine induces its metabolism by CYP3A4/5 and CYP2B6 through the activation of the CAR and PXR nuclear receptors, and polymorphisms in the genes coding for these nuclear receptors influences the expression of the CYPs and hence the therapeutic window of nevirapine plasma drug concentrations in the individual patients [38]. These may result in development of drug toxicity if the enzymes are inhibited, and if overexpressed, they cause subtherapeutic plasma drug concentrations in

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the affected individual. Therefore, sustained ethanol use together with the administered d4T, 3TC and NVP may lead to alcohol-drug interactions that may affect the therapeutic steady-state plasma drug concentrations of the administered drugs to the patients (therapeutic window) [41, 42].

Ethanol, its metabolites like acetaldehyde, ROS including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hypochlorite ion (OCl^-), and hydroxyl (OH^-) radicals that are generated by many reactions in multiple compartments of the cell and as well as other environmental factors like dietary foods, can also trigger the induction of epigenetic (pharmacogenetic) mechanisms such as DNA (de)methylation, histone modifications like (de)acetylation, (de)methylation and (de)phosphorylation as well as the noncoding microRNAs that affects the drug disposition in the body [43–48]. It is also possible that the prolonged use of d4T, 3TC and NVP together with the concurrent use of ethanol and its metabolites, herbs and dietary foods may inhibit the expression of the nuclear receptors such as PXR, CAR, and AhR that activate the CYP450 metabolizing enzyme system especially the CYP3A4/5 and CYP2B6 that mainly metabolize NVP [49, 50], as well as the protein drug transporters of these drugs (d4T and 3TC) thus decreasing their excretion. Metabolites, including those generated during ethanol metabolism, can impact disease states by binding to transcription factors and/or modifying chromatin structure, thereby altering gene expression patterns.

These mechanisms may alter the gene expression that encodes various CYP450 metabolizing enzymes, drug transporter proteins, transcription factors, drug targets and nuclear receptors, thus influencing the individual response to the drug administered [43–46] and leading to drug toxicity or subtherapeutic plasma drug concentrations or alteration of the therapeutic window of the drugs. Also the increased occurrence of the single-nucleotide polymorphisms (SNPs), may influence the individual response to the drugs by affecting the pharmacokinetics and pharmacodynamics of administered drugs and hence either delaying or facilitating the elimination of drugs from the body, thus requiring individual drug dosing consideration (personalized medicine) [23–25]. The sustained use of ethanol by HIV-infected patients on d4T/3TC/NVP drug regimen can activates the epigenetic mechanisms and hence affect the therapeutic steady-state plasma drug concentrations of the administered drugs in the patients. This may lead to either the toxic concentrations of the individual drugs (toxicity) or subtherapeutic drug concentrations (therapeutic failure). In multiple drug dosing such as d4T, 3TC and NVP, after a loading dose the patients are supposed to maintain a therapeutic steady-state plasma drug concentration that has to be maintained during the period of treatment such that at any time of blood sampling and analysis of the plasma drug concentrations, they are maintained within the therapeutic steady-state concentration ranges (Figure 4). The study therefore determined the effect of chronic alcohol use by HIV-infected patients on the therapeutic steady-state plasma drug concentrations (therapeutic window) of d4T, 3TC and NVP drug regimen.

Materials and methods

Study design

A case-control study that used a repeated measures design with serial measurements was used to quantify the plasma drug concentrations of d4T, 3TC and NVP in HIV-infected patients who were exposed to chronic alcohol, and at the same time, they were initiated on the d4T/3TC/NVP drug regimen [triomune 30 (3TC 150 mg, NVP

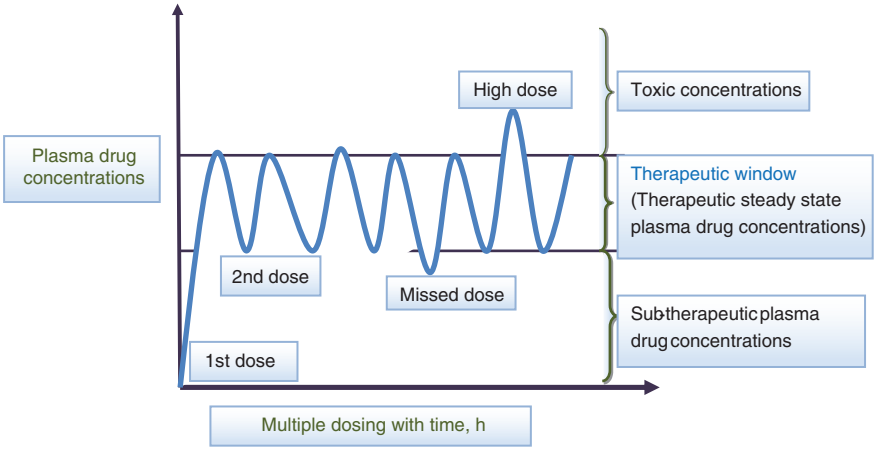


Figure 4 Steady-state plasma drug concentrations following multiple dosing of patients.

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200 mg and d4T 30 mg) tablets] for the last 6 months. The d4T/3TC/NVP drug regimen was selected because during the time of the study it was one of the first-line drug regimens available for the treatment of HIV-infected patients in the country. The serial measurements model was involved in the quantification of the plasma drug concentrations of d4T, 3TC and NVP at 3-month intervals (0, 3, 6 and 9 months) for a period of 9 months for both the control group and the chronic alcohol-exposed group.

Study site and population

The study was conducted at St. Raphael of St. Francis Hospital, Nsambya, a private clinic and at the department of Pharmacology and Therapeutics Pharmacokinetic Laboratory. The hospital handles about 1500 HIV-infected patients. A total of 41 HIV-infected patients who were on d4T/3TC/NVP drug regimen were recruited after being screened for chronic alcohol use using the WHO AUDIT tool and then further sorted using the alcohol-use biomarkers.

Inclusion criteria

The HIV-infected patients who were included in this study were HIV positive and on d4T/3TC/NVP drug combination regimen for the last 6 months at the time of enrollment. They had an adherence rate of ≥95% that was measured using the self-reporting method and pill counts at scheduled visits. This ensured that the patients were taking their medications as per the prescription with no missed doses. Also, those included were in the age range of 18–50 years. For the alcohol group, they were exposed to chronic ethanol at the time of recruitment and during the 9 months of follow-up. In the control group, they were not exposed to any type of alcohol at all or for the last 6–12 months.

Eligibility criteria and enrollment of study participants

The study was conducted on the HIV-infected patients who were all initiated on the d4T/3TC/NVP drug regimen for the last 6 months. A total of 41 HIV-infected patients on d4T/3TC/NVP were screened for chronic alcohol use using the WHO AUDIT tool. Twenty patients (13 males and 7 females) were identified to use ethanol chronically and 21 patients (17 males and 4 females) were identified by the tool as non-alcohol consumers. The WHO AUDIT is a noninvasive method that is routinely used worldwide to screen patients exposed to chronic ethanol [18]. The AUDIT tool has a set of 10 questions, each with responses and scores that the individual responded to by self-reporting. A total score of 8–15 indicates hazardous alcohol use, 16–19 indicates alcohol use problem and scores above 20 indicate alcohol use dependence [18]. All the patients recruited in the chronic alcohol group had a total score of above 8 according to the WHO AUDIT tool interpretation of the scores. The patients enrolled in the control group had a score value of <8. However, because the WHO AUDIT was not sensitive enough to actually detect some of the patients in the control group who were consuming alcohol chronically, chronic alcohol-use biomarkers (GGT, MCV and AST/ALT ratio) were used to further sort out the patients in the control group. The simultaneous elevation of GGT values above 55.0 UI, MCV values above 96 fL and AST/ALT ratio above 2.0 were indicators of chronic alcohol use and these were monitored throughout the 9 months of the study period to ensure that there were no reverts and converts. The baseline serum enzyme concentrations (GGT, ALT and AST) at time 0 month just before they were initiated on the d4T/3TC/NVP drug regimen of all the patients that participated in the study were collected retrospectively from the patients' records to ensure that these patients in the chronic alcohol-use group were being exposed to alcohol. Then, the 41 HIV-infected patients were again grouped according to the chronic alcohol-use biomarkers into two groups. The chronic ethanol use group had 26 patients (22 males and 4 females) and the control group had 15 patients (8 males and 7 females). The patients in both the control and the chronic alcohol-exposed group were followed up for 9 months starting from March 2008 to November 2008. All the patients who participated in the study signed consent forms.

Whole-blood sample collection and plasma processing

About 5 mL of whole venous blood samples from the patients were collected from the cubital vein into a sterile Vacutainer with EDTA as anticoagulant every 3 months for a period of 9 (0, 3, 6 and 9) months. The plasma samples were obtained by centrifuging the blood at 3000 rpm for 5 min. The plasma samples were extracted into clean cryovials and kept into a deep freezer at a temperature of –70°C prior to plasma drug concentration determination.

Plasma drug analysis procedures

The individual d4T, 3TC and NVP plasma drug concentrations were determined using the Shimadzu Class-VP™ HPLC Chromatography data version 6.1 system with UV detector (Shimadzu Corporation Analytical and Measuring Instruments Division, Kyoto, Japan) and using Notari et al.'s method [51].

Chemicals

The pure stavudine, lamivudine and nevirapine that were used as standards were donated by a colleague from the department of Pharmacology and Therapeutics, Gulu University Medical School. Lamivudine was obtained from Iaf Biochem. Int./Glaxo Wellcome (Brentford, Middlesex, UK), nevirapine from Boehringer Ingelheim Pharmaceuticals (Ingelheim, Germany) and stavudine from Bristol-Myers Squibb Pharmaceuticals (New York, NY, USA). All these drugs were of analytical grade and were used as standards and controls in the study. Acetonitrile, methanol and other chemicals used in the study were purchased from BDH Chemicals (Poole, Dorset, UK) and from a distributor representative company in Kampala, Uganda. KH_2PO_4 was donated by a colleague from the Department of Chemistry, Makerere University and was also purchased from BDH Chemicals (Poole, Dorset, UK). All the reagents that were used in the study were of HPLC grade. The distilled water and deionized water were

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produced on site. The blank plasma, free of any drug, was obtained from the Uganda Blood Bank (Nakasero, Kampala, Uganda).

Chromatographic system

The chromatographic system consisted of a Waters 600 pump and a Waters autosample 717 PLUS equipped with a spectrophotometric UV-vis dual-wavelength system Waters 2487 set at 240 and 260 nm (Milford, MA, USA). The drug separation was performed at 24.0°C on an analytical C18 Symmetry™ column (250 mm×4.6 mm I.D.) with a particle size of 5.0 µm (Waters) equipped with a Waters Sentry guard column (20×3.9 mm I.D.) filled with the same packing material (Waters). The Millenium software on the HPLC-UV instrument was used to process the data.

Mobile-phase solutions

The mobile phase was composed of solution A (0.01 M KH_2PO_4) and B (acetonitrile). Both solutions were degassed by sparging with helium. The injection volume was 20 µL. The mobile phase was delivered at a rate of 1.0 mL/min and the gradient elution program was used (Table 2).

Preparation of the stock, working, and blank plasma solutions

Stock solutions of lamividine, nevirapine and stavudine (1.0 mg/mL) were prepared by dissolving 5.0 mg of each of the drug in 5.0 mL of methanol. Stock solutions were appropriately diluted with methanol for the preparation of working standard solutions (final concentrations ranging between 0.005 and 10 µg/mL). The stavudine, lamivudine and nevirapine drug concentration in blank plasma calibration samples ranged between 0.005 and 10 µg/mL. The blank plasma was used to mimic the patient plasma samples.

Sample preparation

The plasma samples that were extracted from the HIV-infected patients on d4T/3TC/NVP drug combination regimen were cleaned up by offline solid-phase extraction (SPE) using Oasis HLB Cartridge 1 cc (30 mg) (Waters). The SPE cartridges were conditioned with 1.0 mL of methanol followed by 1.0 mL of water (Milli-Q). One hundred microliters of methanol was added to 600 µL of human plasma, the solutions were vortexed for 1.0 min and then centrifuged at 5200 rpm for 15.0 min at 24.0°C. The supernatant (ca. 650 µL) was diluted by adding water (Milli-Q) (1.0 mL) and loaded in the cartridge. The cartridges were washed with 1.0 mL of 5% (v/v) methanol in water (Milli-Q). The analytes were eluted by washing cartridges with 550 µL of 0.01 M KH_2PO_4 followed by 2.0 mL of absolute methanol. The eluate was evaporated in a water bath at 36.0°C under a stream of nitrogen. The extracted sample was reconstituted with 100 µL of absolute methanol and transferred to an injection vial. The HPLC-UV detection at 240 and 260 nm and the gradient program was used for stavudine, lamivudine and nevirapine drug separation and concentration determination for each drug.

Calibration curves and recovery

The calibration curves were established over the 0.05, 0.1, 0.5, 1.0, 5.0 and 10 µg/mL concentration ranges for lamivudine and nevirapine and 0.1, 0.5, 1.0, 5.0 and 10 µg/mL concentration ranges for stavudine. The absolute recovery of each of these drugs from plasma was obtained as the peak area response of the processed samples, expressed as the percentage of the response of the drugs contained in the 20-µL injection volume and not subjected to SPE. The average retention time used for d4T, 3TC and NVP drugs are shown (Table 3). The areas of the chromatogram peaks for the standards were used to make a standard calibration curve for each drug (Figure 4). The concentration of each drug in each patient's plasma sample was obtained by using the generated equation of the straight line and the slope. Microsoft Excel was used to prepare the standard curve and the calculation of the plasma drug concentrations of each drug in each plasma sample using the slope, intercept and the generated area of the chromatogram peak for each sample. The results of the plasma drug concentrations were compared with reference values obtained from the literature.

Data management and quality assurance

All the samples from the patients were coded to ensure privacy and confidentiality throughout the study period. All the reagents used were of HPLC grade. All the samples analyzed were replicated. Standard operating procedures were used throughout the drug analysis in the laboratory. All the data collected and analyzed were entered in the Microsoft Excel spreadsheet for all the patients and was cleaned. The results were presented in the form of tables and line graphs.

Table 2 Gradient elution program.

Time, min	Flow, mL/min	KH_2PO_4 solution A, %	Acetonitrile solution B, %	pH
0	1	94	6	5.0
10	1	30	70	4.5
15	1	94	6	4.5
20	1	94	6	4.5
25	1	0	100	–

Table 3 Average retention time of d4T, 3TC and NVP drugs used.

ARV drug	Average retention time
Lamivudine	5.02
Stavudine	6.3
Nevirapine	16.5

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Data analysis

All the data were entered in the Microsoft Excel spreadsheet was then imported into the SAS 2003 version 9.1 statistical package for statistical data analysis (Cary, NC, USA). The data were analyzed at 95% confidence interval. The repeated measures fixed model was used during the statistical data analysis. The t-test was used to compare the means of the steady-state plasma drug concentrations in the chronic alcohol-use group and the control group at different time intervals. The outcome measures were the mean difference in the steady-state plasma drug concentrations of each drug in the chronic alcohol-use group and control group basing on the chronic alcohol-use biomarkers. A value of $p \leq 0.05$ was considered as statistically significant.

Ethical consideration

The research work was approved by the Faculty of Medicine Higher degrees, Research and Ethics Committee of the Makerere University Institution Review Board (IRB) (IRB#-2007-060), IRB of St. Raphael of St. Francis Hospital, Nsambya (no. IRB 03: 01/03/2008) where the study participants were recruited from and the Uganda National Council for Science and Technology (UNCST) (no. HS 387). In this study, a written informed consent was obtained from each human subject and all procedures used were in accordance with ethical standards of the responsible committee on human experimentation (institutional or regional) and in accordance with the Helsinki Declaration of 1975, as revised in 1983. They were given study code numbers, which were used throughout the study period in order to protect their privacy and confidentiality.

Results

The effect of chronic ethanol use on the therapeutic steady-state plasma drug concentrations of d4T, 3TC and NVP in the HIV-infected patients on d4T/3TC/NVP drug regimen was determined during the 9-month period of follow-up. Figure 5 shows the chromatogram of the d4T, 3TC and NVP drug analysis in plasma samples of the patients. The results show that the mean d4T and 3TC therapeutic steady-state plasma drug concentrations were higher than the reported therapeutic steady-state plasma drug concentration reference ranges of 0.09–0.68 and 0.2–1.42 μg/mL, respectively (Table 1). The d4T plasma concentrations in the chronic alcohol-use group were lower in the 3, 6 and 9 months than in the control group during the follow-up period but the difference was statistically insignificant ($p \geq 0.05$). However, in the 6-month follow-up, the mean d4T plasma drug concentrations in the control group were observed to increase as compared to the chronic alcohol-use group (Table 4 and Figure 6). The 3TC mean plasma drug concentration in the alcohol-use group was higher than in the control group at 3 and 6 months of follow-up, and the difference was significant ($p \leq 0.05$) at 6 and 9 months between the two groups. The mean NVP plasma drug concentrations in both groups were within the normal therapeutic steady-state plasma drug concentrations (5.3–11.7 μg/mL) (Table 1) except in the 9-month follow-up of the control group where it was 3.91±1.32 μg/mL. The mean NVP plasma drug concentrations in the chronic alcohol-use group were higher than in control group throughout the 9-month period of follow-up. The difference in the steady-state NVP plasma drug concentrations in the chronic alcohol group and the control group was statistically significant ($p \leq 0.05$) at 0, 3, and 6 months of follow-up (Table 4). It was also observed that at the 6-month follow-up, the d4T, 3TC and NVP concentrations in the control group tended to increase, whereas in the chronic alcohol-use group the concentrations decreased in the same month after which they reverted to the normal trend (Figure 6).

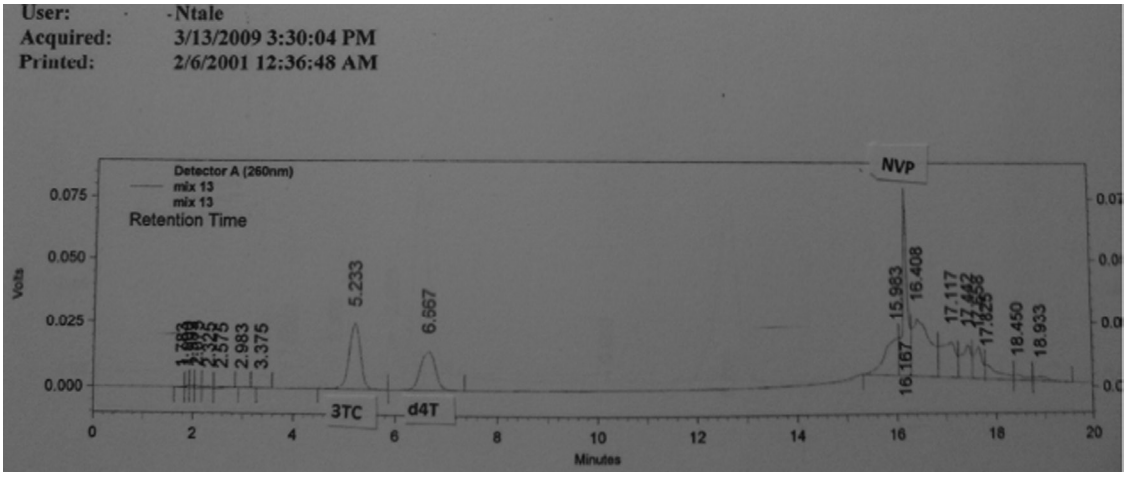


Figure 5 Chromatogram of d4T, 3TC and NVP.

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Table 4 Mean d4T, 3TC and NVP plasma drug concentrations in the HIV-infected patients during the 9 months follow-up period.

Mean plasma drug concentrations	Time of follow-up, months				Therapeutic window
	0	3	6	9	
d4T±SE, µg/mL					
Control	0.70±0.50	1.00±1.22	4.45±1.84	3.62±2.50	0.09–0.68
Alcohol	0.92±0.97	0.70±0.32	1.04±0.92	0.98±1.11	
p-Value	0.15	0.78	0.54	0.13	
3TC±SE, µg/mL					
Control	3.32±2.26	3.83±3.02	4.45±1.84	3.79±2.50	0.2–1.42
Alcohol	3.74±1.92	3.37±1.13	3.78±1.73	4.51±3.63	
p-Value	0.13	0.16	0.04	0.01	
NVP±SE, µg/mL					
Control	5.69±2.50	5.56±1.72	5.42±1.76	3.91±1.32	5.3–11.7
Alcohol	6.37±2.72	6.39±3.08	6.54±3.29	5.86±2.25	
p-Value	0.03	0.004	0.001	0.09	

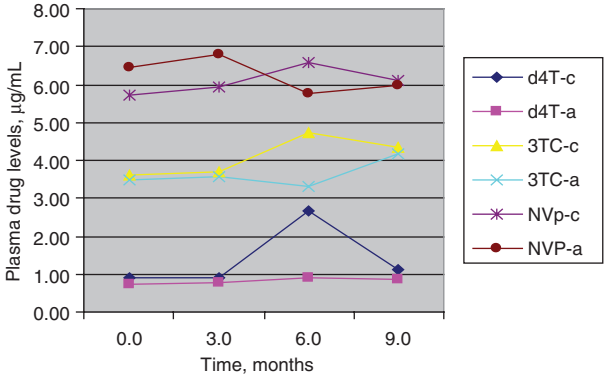


Figure 6 Variation of the mean d4T, 3TC and NVP steady-state plasma drug concentrations with time for both chronic alcohol-use and control groups.
d4T-c, d4T in control group; d4T-a, d4T in chronic alcohol; 3TC-c, 3TC in control; 3TC-a, 3TC in chronic alcohol; NVP-c, NVP in control; NVP-a, NVP in chronic alcohol.

Discussion

The effect of chronic ethanol use on the steady-state plasma drug concentrations of d4T and 3TC were above the therapeutic steady-state plasma drug concentration reference ranges for both the chronic alcohol-use and the control groups. This may possibly be due to the pharmaceutical problem during the process of compounding of the drugs in which more active ingredients may be incorporated into the drug that may be higher than that recommended in standard pharmacopoeias (USP, BP and IP) [52, 53]. Also, because the patients were on prolonged highly active antiretroviral treatment (HAART) treatment, the observed high steady-state plasma drug concentrations of d4T and 3TC could be due to the liver and the kidney damage associated with the drugs' toxicities, and this delays the elimination of the drugs from the body, leading to their accumulation in the blood circulation of the patients. These organs are responsible for the elimination of these drugs from the body, the process that could have been compromised by the drug toxicities to the organs [26, 32, 54, 55]. Also, the observed high steady-state plasma drug concentrations of d4T and 3TC in some patients could also be due to the suppressed epigenetic mechanisms by the drugs themselves (nucleoside and non-nucleoside analogues that are DNA methyltransferase inhibitors) and as well as exposure to other environmental factors like alcohol, herbs and dietary substances by the individual patients that causes failure of gene expression that encodes for the CYP450 drug-metabolizing enzymes as well as failure of drug transporters from blood circulation to tissues and their eventual elimination from the body [43–45, 47, 48, 56]. It is also possible that the prolonged use of d4T, 3TC and NVP together with the concurrent use of alcohol and its metabolites, herbs and dietary foods may inhibit the expression of the nuclear receptors such as PXR, CAR, and AhR that activate the CYP450 metabolizing enzyme system, especially the CYP3A4/5 and CYP2B6 that mainly metabolize NVP [49] as well as the protein transporters of these drugs (d4T and 3TC) for excretion. This therefore reduces the metabolism and excretion of the administered drugs from the body and hence the observed high steady-state plasma drug concentrations of d4T and 3TC in the patients. In addition, single-nucleotide polymorphisms (SNPs) of which about 15 million SNPs have been identified in the human genome, many of which affect gene function in promoters or in cis- or trans-regulatory elements by modifying sequences of proteins or nucleic acids that then affect the translation and transcription processes leading to the inactivation of CYP450 enzyme

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systems and drug protein transporters, thus leading to the failure of expression of CYP450 enzyme systems and drug transporter proteins [57], and this may have contributed to the observed high d4T and 3TC plasma drug concentrations as a result of suppressing the CYP450 and drug transporter systems and delaying the elimination of the drugs from the body [23–25, 56]. These factors may be the reason why most of these patients present with a number of adverse effects associated with each of these drugs.

For NVP, the steady-state plasma drug concentrations in the HIV-infected patients in both the chronic alcohol and the control groups were within the reported therapeutic steady-state plasma drug concentration ranges except in the 9th month of follow-up in the control group (Table 4). The NVP has a wide therapeutic window, and therefore it is less likely to be affected by minimal exposure to environmental factors like alcohol as compared to d4T and 3TC. The low steady-state plasma drug concentrations of NVP below the reference ranges in the 9 months in the control could be due to the CYP450 isoenzyme autoinduction that promotes the elimination of the drug from the body. However, the mean steady-state plasma NVP drug concentration was higher in the chronic alcohol-use group as compared to the control group throughout the 9 months of follow-up period, and it was statistically significant at 0, 3, 6 months ($p=0.03$, $p=0.004$, $p=0.001$), respectively. The high plasma drug concentrations in the chronic alcohol group could be due to the alcohol metabolites such as acetaldehyde, the ROS generated that may have played a significant role in suppressing the gene expression for the CYP450 isoenzymes responsible for the metabolism of NVP and the drug transporters that facilitate the elimination of the drug from the body [23–25, 56]. Therefore, in this study, chronic ethanol use by the HIV-infected patients on d4T/3TC/NVP drug regimen affected the therapeutic steady-state plasma drug concentrations of d4T, 3TC and NVP in the patients during the 9 months of the study period of follow-up.

Conclusions

Chronic ethanol use by the HIV-infected patients on d4T/3TC/NVP drug regimen affects the therapeutic steady-state plasma drug concentrations or therapeutic window of d4T, 3TC and NVP in the patients during the 9-month study period of follow-up. The accumulation of ethanol and its metabolites in the body as well as the prolonged use of d4T, 3TC and NVP damages the liver and the kidney, which are responsible for the elimination of the drugs from the body, thus increasing the plasma drug concentrations in the blood circulation of the patients as observed in the study. The patients in the study were observed to maintain the NVP therapeutic steady-state plasma drug concentrations in their blood circulation all throughout the study period of follow-up except in the 9 months in the control group. Also, ethanol and its metabolites as well as the d4T, 3TC and NVP drugs, the patients were taking could activate or suppress the nuclear receptors such as CAR, PXR and AhR that code for the various CYP450 enzyme isoforms and drug transporters, thus affecting the rate of metabolism and excretion of the drugs from the body. This eventually leads to the individual variations in the therapeutic steady-state plasma drug concentrations observed in the different HIV-infected patients in the study. Therefore, in this study chronic ethanol use by the HIV-infected patients on d4T/3TC/NVP drug regimen affected the therapeutic steady-state plasma drug concentrations of d4T, 3TC and NVP in the patients during the 9 months of study period of follow-up.

Acknowledgments: The following are acknowledged for their contribution to this work. We thank Mr. Gordon Ewa in the Department of Pharmacology and Therapeutics, MaCHS, for assistance in lab work; Sr. Justine Birungi, Sr. Plaxeda, Sr. Namugosa, Sr. Jesca and Dr. Kayima from St. Francis Hospital, Nsambya, for recruitment of subjects and collection of blood samples from the patients; the Director of St. Raphael of St. Francis Hospital, Nsambya, and Dr. Pius Okong, chairman of the IRB for allowing the study to be conducted in the hospital; and Dr. Norah Mwebaza in the Department of Pharmacology and Therapeutics, MaCHS, for scientific support.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

Received July 12, 2013; accepted November 18, 2013; previously published online January 27, 2014

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