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Chronic binge alcohol consumption does not diminish effectiveness of continuous antiretroviral suppression of viral load in SIV-infected macaques

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Abstract

Background—Alcohol use disorders (AUD) are a frequent comorbidity in a large percentage of people living with HIV/AIDS (PLWHA). PLWHA with comorbid AUDs are consistently found to perform poorly at most levels of the HIV treatment cascade, resulting in a higher likelihood of virologic non-suppression. This has been partly attributed to lower rates of persistence with and adherence to antiretroviral therapies (ART). Focus groups of in-care PLWHA identify the need to suspend ART on drinking days because of the potential for toxicity and/or lack of therapeutic effectiveness. The aim of this study was to examine whether chronic binge alcohol (CBA) consumption decreases the effectiveness of uninterrupted ART, specifically that of nucleoside reverse transcriptase inhibitors (NRTI) tenofovir and emtricitabine in suppressing viral replication, or results in drug toxicity in simian immunodeficiency virus (SIV) infected rhesus macaques.

Methods—Daily CBA or isocaloric sucrose (SUC) administration was initiated three months prior to intrarectal SIV_{mac251} inoculation and continued throughout the study period. ART was initiated 2.5 months after SIV infection and continued through the study period.

Results—CBA administration did not prevent or delay the ART-mediated reduction in viral load. Following ART, circulating levels of total protein and creatinine were significantly higher than baseline values in both sucrose- and alcohol-treated animals, but still within a normal range. No evidence of ART toxicity was observed in either CBA- or SUC-administered macaques.

Conclusions—These findings indicate that CBA does not attenuate effectiveness of NRTI suppression of viral load, nor does it appear to interact with NRTI to produce toxicity during the initial 2 months of treatment. We conclude that while efforts to reduce AUD in PLWHA should be a priority, and that counseling on the importance of adherence to ART even on drinking days should also be promoted.

Keywords

rhesus; alcohol; simian immunodeficiency virus; antiretroviral; toxicity

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Introduction

According to the Center for Disease Control, more than 400,000 people were living with HIV/AIDS (PLWHA) in the US at the end of 2003 (Center for Disease Control and Prevention, 2012). More than 50% of the adult population in the United States reports alcohol consumption, with 6% of the adult population fulfilling criteria for heavy drinking, and 22% for binge drinking (Substance Abuse and Mental Health Services Administration, 2012). The substantial reduction in HIV-associated morbidity and mortality, resulting from the use of anti-retroviral therapy (ART) has made HIV infection a chronic disease during which individuals are likely to engage in alcohol and drug abuse at rates comparable or higher than those of the non-infected population (Galvan et al., 2002, Lefevre et al., 1995).

Adherence to ART is strongly associated with virologic suppression, immunologic improvement, decreased progression to AIDS, and control of drug resistance (Lima et al., 2008). Alcohol use disorders (AUD) interfere with adherence to ART (Azar et al., 2010). PLWHA that have AUDs are consistently found to perform poorly at multiple levels of the HIV treatment cascade, resulting in a higher likelihood of virologic non-suppression (Gardner et al., 2011, Chander et al., 2006, Palepu et al., 2003). Large observational studies have shown that hazardous alcohol consumption decreases overall survival in PLWHA in what appears to be a dose-dependent manner (Brathwaite et al, 2007). Frequent alcohol consumption has been reported to result in higher likelihood of presenting a decline of CD4 to 200 cells/ μ l in PLWHA (Baum et al., 2010). In addition, alcohol consumption is a significant predictor of non-adherence to ART (Parsons et al., 2008, Samet et al., 2004) and this has been shown to result in worse response to treatment (Lima et al., 2014). Clinical studies indicate that up to 50% of HIV+ patients report skipping or stopping ART when drinking. The underlying cause for lack of adherence in HIV+ with AUD is not well understood (Hendershot et al., 2009). Associated depressive symptomatology (Kalichman et al., 2013), patient's belief that ART and alcohol should not be mixed (Sankar et al., 2007, Kalichman et al., 2013), forgetfulness (Cook et al., 2001), and neurocognitive impairment (Hinkin et al., 2004) have been proposed to be some of the causal factors for non-adherence in this patient population.

The possibility that chronic alcohol consumption may alter effectiveness of ART has not been examined in a controlled systematic way. Moreover, whether a pattern of chronic binge alcohol consumption interferes with effective viral suppression or produces toxicity is not fully understood. A controlled study of the impact of chronic alcohol abuse in PLWHA is virtually impossible to conduct in a clinical setting. Among the multiple confounding factors are the difficulty in selecting a homogenous patient population and the confounding effect of prescription, experimental and illicit drugs frequently used by these individuals. The non-human primate (NHP) model of immunodeficiency infection circumvents these limitations, allowing a controlled study design to identify the impact of chronic alcohol administration

on ART effectiveness in control of viremia. The inoculation of rhesus macaques with simian immunodeficiency virus (SIV) is recognized as the best animal model for studying the pathogenesis of HIV-like infection (Gardner and Luciw, 1988, Letvin and King, 1990) and has been used by our group to dissect the impact of chronic binge alcohol consumption on disease progression, response to bacterial infections, and nutritional and musculoskeletal alterations leading to muscle wasting (Bagby et al., 2003; Molina et al, 2006 and 2008). Experimental SIV infection of rhesus macaque results in a disease that is remarkably similar to human AIDS (Arthur et al., 1986) characterized by a marked reduction in CD4⁺ cells and in the CD4⁺/CD8⁺ cell ratio. Using this clinically-relevant model, we investigated whether in the presence of strict adherence to ART, CBA consumption impacts effectiveness of viral suppression. In addition, we examined whether CBA consumption produced significant alterations in circulating levels of biochemical markers for liver and renal toxicity during the initial 2 month phase of uninterrupted ART. The combination of ART used in the study consisted of two reverse transcriptase inhibitors, tenofovir and emtricitabine, which have previously been demonstrated to reduce viral load in NHP (Lewis et al., 2010, Lifson et al., 2003, Verhoeven et al., 2008). This drug combination, (also known as Truvada) is widely prescribed in combination ART regimens and has recently been approved for use as a pre-exposure prophylactic (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2014, Center for Disease Control and Prevention, 2012, Center for Disease Control and Prevention, 2011)

Materials and Methods

All experiments were approved by the Institutional Animal Care and Use Committee at both the Tulane National Primate Research Center (TNPRC) in Covington, LA, and Louisiana State University Health Sciences Center in New Orleans, LA and adhered to the NIH guidelines for the use of experimental animals. Three to six year old healthy male rhesus monkeys (*Macaca mulatta*) obtained from the breeding colonies at the TNPRC were studied as previously described. Animals were individually housed in a Biosafety Level-2 (BSL-2) containment building.

Experimental protocol

Twenty four age- and body weight-matched animals were randomized first to either chronic binge alcohol (CBA) or isocaloric sucrose (SUC) treated groups. Three months after initiating the CBA or SUC administration protocols, animals were infected intrarectally with simian immunodeficiency virus (SIV_{mac251}) as detailed below. Approximately two and a half months after SIV inoculation, animals were further randomized to ART+ or ART- groups. ART was continued throughout the study period. The data reported reflect the period of infection prior to and during the initial 2 months after initiation of ART. Thus, four experimental groups (N=6 per group) were studied: CBA/SIV/ART+; CBA/SIV/ART-; SUC/SIV/ART+; and SUC/SIV/ART-.

Daily CBA (or SUC) administration was initiated three months prior to SIV inoculation and continued throughout the duration of the study, with the exception of the days when biological sampling was performed, as previously described (Bagby et al., 2003). Briefly,

animals were fitted with a gastric catheter and catheter-protecting jacket and tether attached to a swivel that allowed animals to move about their cages during alcohol administration. CBA consisted of daily ethanol (~13 to 14 g ethanol/Kg body weight/wk; 30% w/v water) administered via the chronically-fitted intra-gastric catheter to ensure maximal control of the amount of alcohol animals received throughout the course of the study. This approach of intra-gastric delivery over voluntary alcohol consumption was selected to reduce experimental variability and ensure chronic binge-like intoxicating blood alcohol concentrations between 50–60 mM as previously reported. Chronic binge alcohol consumption was chosen as a model to examine hazardous drinking patterns frequently observed in PLWHA. Though not perfect in replicating AUD it does represent the most frequent pattern of alcohol abuse and is associated with numerous comorbid conditions (CDC). Blood alcohol levels were measured on a weekly basis at 2 h after starting the “binge” protocol. Adjustments that did not vary between ART+ and ART– animals were made to ensure alcohol concentrations remained within the 50–60 mM range. Time-matched control monkeys were subjected to the same surgical procedures, but received an isocaloric SUC infusion. Total calories provided by alcohol and sucrose averaged 15% of daily intake. Animals were provided ad libitum with Monkey chow (Lab Fiber Plus Primate diet-DT, PMI Nutrition International, St. Louis, MO) and supplemented with fruits, vitamins and Noyes’ treats (Research Diets, New Brunswick, NJ).

Three months after initiating CBA or SUC administration, animals were inoculated intrarectally with 100 TCID₅₀ (50% tissue culture infectious doses) of SIV_{mac251} provided by Dr. Preston Marx at the TNPRC (Ling, 2002). Inoculation was performed at the conclusion of an alcohol or sucrose session to simulate infection during an alcohol-binge episode. The progression of SIV disease was monitored throughout the study period through clinical and biochemical parameters including complete blood count (CBC) and enumeration of lymphocyte subsets, serum chemistries (protein, albumin, globulin, alanine aminotransferase (ALT), amino aspartate aminotransferase (AST), alkaline phosphatase (ALK) and plasma SIV gagRNA. Serum chemistries were determined using a Cobas Mira Chemistry Analyzer (Roche, Rotkreutz, Switzerland) at the Clinical Chemistry Laboratory at the TRPRC. Table 1 summarizes the range of normal values as well as the range of values obtained from animals in the present study. Serum globulins were calculated as the difference between total serum protein and albumin. Clinical variables monitored included: body weight and temperature, general physical condition, presence of lymphadenopathy, splenomegaly, presence of rashes or skin abnormalities, condition of oral and conjunctival mucosa, diarrhea and signs of opportunistic infections. All parameters were assessed on a non-alcohol day following an overnight fast.

Antiretroviral therapy

ART consisted of tenofovir (PMPA, 9-R-2-phosphonomenthoxypropyl) adenine and emtricitabine (FTC, beta-2, 3-dideoxy-3-thia-5-fleirocytidine). ART was injected subcutaneously at a dose of 30 mg/kg/day each. ART– animals were injected with equal volumes of vehicle. This ART protocol was selected based on published reports of their efficacy in reducing viral load (Verhoeven et al., 2008, García-Lerma et al., 2010). Both drugs are nucleoside reverse transcriptase inhibitors (NRTI), which suppress replication of

retroviruses by interfering with the reverse transcriptase enzyme. This drug combination has been shown to be effective in control of viral load and to result in minimal toxicity in normal healthy rhesus macaques from infancy to adulthood and pregnancy, even after prolonged administration (Van Rompay et al., 2008). Tenofovir has been reported to produce renal toxicity when administered at high doses (Valle and Haragsim, 2006). Because of this, tenofovir was reduced to 20 mg/kg after the first 2 months of drug treatment.

Statistical analysis

All response variables were analyzed using a linear repeated measures design model. The repeated factor was time measured in months post SIV inoculation (MPSIV). The observations over time were modeled using an autoregressive type I covariance structure to account for dependences between observations on the same animal at different time points. The fixed effects included the CBA/SUC, ART +/-, and MPSIV main effect. All two-way and three-way interactions were included in all models. Coding for ART +/- treatment main effect took into account the random assignment of animals to ART +/- treatment at 2.5 MPSIV. Outcome measures for which the pre-CBA or SUC administration values were available were included in the model as an additive covariate in order to adjust for baseline levels. For all test statistics testing hypotheses about main effects, interactions, and multiple comparisons, we used the F-distribution approximation with the Kenward-Roger type degrees of freedom. The adjusted p-values with respect to Tukey-Cramer adjustment are reported. Analysis was performed by Proc Mixed in SAS Version 9.3 statistical software package.

Results

Viral load and immune function markers

No significant difference in plasma viral load was observed between the animals administered CBA or SUC at any of the time points studied (Figure 1). Both SUC and CBA animals showed similar viral kinetics over the initial 10 weeks of infection. Following the initiation of ART, both SUC/SIV/ART+ and CBA/SIV/ART+ groups had significantly lower mean viral load after 2 weeks of ART (3 MPSIV) as compared to viral levels observed prior to ART initiation, which continued throughout the study period (CBA; LS means difference 5.97, stderr=0.84, $p<0.0001$ and SUC; LS means difference 3.99, stderr=0.913, $p=0.02$ respectively). Suppression of plasma viral load did not differ between SUC/SIV/ART+ and CBA/SIV/ART+ groups.

No significant difference in mean lymphocytes counts was observed between SUC- and CBA-administered groups. However, in the CBA/SIV/ART+ group we observed a significant increase in lymphocyte counts at 3.5 MPSIV compared to pre-ART values (2.5 MPSIV) ($F(1,48.2)=6.61$, $p=0.01$). As expected, SIV infection resulted in a significant decrease in %CD4 lymphocytes and CD4+ to CD8+ lymphocyte ratio in both SUC/SIV and CBA/SIV groups (Figure 2B and 2C). However, no differences in mean %CD4 lymphocytes or CD4+/CD8+ lymphocytes ratio were noted between CBA- and SUC-administered animals throughout the study period. Following ART, % CD4 lymphocytes in both SUC/SIV/ART- and CBA/SIV/ART- animals, continued to decline, and remained

significantly lower than pre-SIV values at 3.5 and 4 MPSIV ($p < 0.5$). Over the period of ART, levels of % CD4 lymphocytes in SUC/SIV/ART+ and CBA/SIV/ART+ remained relatively stable as compared to pre-ART values and were not significantly different from pre-SIV values.

Hematology and blood chemistries

No significant difference in platelet count was detected between SUC- and CBA-administered animals in either the ART+ or ART- groups throughout the study period (Figure 2D). However, in the CBA/SIV/ART- animals, a significant decrease in mean platelet count from pre-SIV values was observed at 3.5 and 4.5 MPSIV. No significant differences in mean hemoglobin or hematocrit values were detected between SUC- and CBA-administered animals either in response to SIV infection or ART treatment at any of the time points studied (data not shown). Values for all animals were within the normal range provided by the clinical laboratory (Table 1).

Serum proteins

Total serum protein levels showed a transient and significant decrease in the SUC/SIV animals at 0.5 MPSIV (LS means difference = 0.566 g/dl, $\text{stderr} = 0.143$, $p = 0.017$) that was restored to levels higher than pre-SIV values at 3.5 MPSIV (LS means difference is 0.998 g/dl, $\text{stderr} = 0.226$, $p = 0.0037$) (Figure 3A). We did not detect a significant change in mean total protein between time points in the CBA/SIV/ART+ or CBA/SIV/ART- groups. For albumin, we detected a statistically significant main time effect (MPSIV), ($F(5,89.2) = 4.96$, $p = 0.0005$) as well as a CBA/SUC-by-ART+/ART- interaction effect on the mean response ($F(1,86.8) = 5.09$, $p = 0.027$). Importantly, no significant differences between the CBA- and SUC-administered animals, with and without ART were observed (data not shown). Furthermore, no significant change in mean response between time points in either treatment group was noted; with the exception of a short-lived decrease from pre-SIV at 0.5 MPSIV in the SUC-administered group (LS means difference is 0.347 g/dl, $\text{stderr} = 0.09$, $p = 0.02$). No differences in treatment main effects, or interaction effects were noted on mean albumin to globulin ratio response, or change over time in either of the treatment groups (data not shown).

Cholesterol and triglycerides

Except for the pre-ART treatment period at 0.5 MPSIV when we observed a significantly higher mean cholesterol level in the CBA- compared to SUC-administered group (LS means difference of 21.2 mg/dl with $\text{stderr} = 8.29$, $p = 0.01$ unadjusted) we did not observe a significant CBA vs. SUC effect at any of the time points for ART+ or for ART- groups. In the CBA-administered animals, a significant ($\text{stderr} = 5.2$, $p < 0.0001$) yet transient increase (still within normal range for the clinical laboratory) in mean cholesterol levels (LS mean 31.25 mg/dl) was observed at two weeks post-SIV inoculation (Figure 3b). However, at 2.5 MPSIV (pre-ART) the mean cholesterol level had returned to pre-SIV levels. CBA/SIV/ART+ animals showed significantly decreased mean cholesterol levels after 2.5 MPSIV. No significant differences in cholesterol levels were observed between SUC/SIV/ART- and SUC/SIV/ART+ groups. A significant time (MPSIV) main effect on the mean response ($F(5,52.4) = 5.25$, $p = 0.0006$) was observed for triglycerides. No difference in

mean response between different levels of treatments or between time points within treatment groups was observed.

Liver and renal function assessment

To assess liver function, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALK) were determined throughout the course of the study. Important to note is that all values measured were within the normal range provided by the clinical laboratory. A transient, yet not statistically significant rise in AST levels was observed at 0.5 MPSIV in both SUC- and CBA-administered animals (Figure 4A). Mean AST values were significantly higher in the CBA/SIV/ART- than in time-matched treatment control animals (LS means difference is 16.95U/L, stderr=8.515, $p=0.049$ unadjusted) at 4 MPSIV. ALT and GGT levels were variable throughout the study period yet remained within a normal range (Table 1). No significant differences in mean ALT or GGT were detected between the SUC- and CBA-administered groups at any of the time points examined (data not shown). SIV infection resulted in a significant decrease in ALK in SUC-administered animals (LS means difference is 106.33U/L, stderr=23.03, $p<0.002$ at 0.5 MPSIV; and LS mean difference 102.9U/L, stderr=27.5, $p=0.03$ at 2.5 MPSIV). CBA-administered animals had significantly lower ALK levels than those of SUC-administered animals at pre-SIV and 0.5 MPSIV, and these were not altered following SIV infection (Figure 4B). At 4.5 MPSIV, mean ALK levels in the SUC/SIV/ART+ were higher than those in the SUC/SIV/ART- group (LS means difference is 229.64, stderr=48.85, $p=0.002$), and those in CBA/SIV/ART+ animals (LS means difference is 225.57 U/L, stderr=52.15, $p=0.005$).

To monitor possible alterations in renal function reflecting tenofovir nephrotoxicity, we determined blood urea nitrogen, creatinine and phosphorus levels throughout the study period. No significant main effects or interaction effects on the mean response, or differences in mean response between treatment groups or between time points were noted for blood urea nitrogen (data not shown). Furthermore, our results do not show any significant differences in mean creatinine levels between SUC- or CBA-administered animals at any of the time points in either ART+ or ART- groups. CBA/SIV/ART+ animals showed a significant increase in mean creatinine levels at 3.5 MPSIV (Figure 4C). However, values remained within the normal range as depicted in Table 1. Significantly higher mean phosphorus levels were observed in the CBA-administered group than in SUC-administered animals at 0.5 MPSIV (LS means difference is 0.922, stderr=0.413, $p=0.029$, unadjusted) (Figure 4D). No significant differences between groups were noted thereafter.

Discussion

We examined the impact of CBA consumption on the effectiveness of ART in reducing viral load in SIV-infected macaques. Our results show that the two NRTI ARTs used in this study produced similar suppression in viral load observed by others (Lewis et al., 2010, Lifson et al., 2003, Verhoeven et al., 2008) and that CBA-treatment did not alter this suppression compared to control animals receiving sucrose. In addition, our findings suggest that under conditions of adequate nutritional intake, strict adherence to ART, and the absence of

additional comorbid conditions, no significant adverse or toxic reactions to ART are noted in CBA-consuming animals.

Heavy alcohol use in people living with HIV/AIDS (PLWHA) is common and has been reported to exceed that of the general population (Galvan et al., 2002). Alcohol commonly impacts HIV disease management by way of decreased adherence to treatment regimens. In fact, multiple studies have demonstrated that recent heavy alcohol use is among the strongest predictors of antiretroviral non-adherence in PLWHA (Do et al., 2013, Hendershot et al., 2009, Cook et al., 2001, Grierson et al., 2011, Glass et al., 2010). While previous studies have focused primarily on alcohol's impact on adherence by way of alcohol-induced cognitive impairments (i.e., unintentional non-adherence), there is emerging evidence suggesting that a subset of patients are intentionally non-adherent as well (Hendershot et al., 2009). A recent study by Kalichman and colleagues (Kalichman et al., 2012) reported that a substantial number of PLWHA, approximately one half in the study sample, either skip medications when drinking or plan to temporarily discontinue ART if they know they will be drinking. PLWHA who reported intentional non-adherence were more likely to endorse beliefs about the toxicity of alcohol and ART interaction. Given these findings, a comprehensive understanding of the relationship between alcohol and ART toxicity and dissemination of this knowledge is necessary to provide PLWHA with the information to make informed decisions that impact disease management.

The results from the current study provide insight into the impact of chronic binge-like alcohol consumption on a clinically relevant model of HIV infection. Daily alcohol administration sufficient to intoxicate animals did not alter the efficacy of combined PMPA and FTC treatment on reducing plasma viral load. Also, differences in CD4 T cell counts were not observed. The daily alcohol delivery protocol used in this study models a chronic binge, the most frequent pattern of alcohol abuse in the general population, and one frequently observed in PLWHA (Jacob et al., 2013). Generalization of these findings is limited because of diverse dose- and time-dependent effects of patterns of alcohol consumption, and the use of different ART drug combinations amongst PLWHA. Additional studies will be needed to address these two issues. Importantly, clinical studies have identified a potential link between poor ART efficacy and adherence to ART, but do not rule out drug effectiveness in HIV infected patients with AUD. Cross-sectional studies have associated both moderate and at-risk drinking among HIV-infected people taking ART with higher viral loads and lower CD4+ T-cell counts as compared to non-drinkers (Miguez et al., 2003; Wu et al., 2011). Others have shown that increased viral loads among people reporting alcohol consumption is associated with suboptimal adherence to ART (Samet et al., 2003). Similarly, a longitudinal study evaluating the impact of alcohol use on HIV disease observed increased viral loads and reduced CD4+ T-cell levels among those with frequent alcohol use to be associated with reduced adherence to ART (Baum et al., 2010). These observations, taken together with the ART efficacy observed in this study, indicate that strategies to improve ART adherence are needed for PLWHA with an AUD.

SIV infection resulted in similar marked decreases in blood %CD4 lymphocytes and CD4+/CD8+ ratios in both SUC- and CBA-consuming macaques as previously described (Bagby et al., 2003). While a robust ART-mediated restoration of CD4 lymphocytes was not evident

in our study, in agreement with reports from others (Smith et al., 2000), an apparent stabilization of %CD4 lymphocytes was seen in ART+ animals in contrast to ART– animals. It is possible that a significant increase in CD4 counts could have been detected at later time points, as reported by others (George et al., 2005). Reports on the response of CD4 lymphocytes to ART indicate a wide range of responses that may not necessarily correlate with the robust suppression of viral load (Van Rompay et al., 2004). The possibility that CD4 lymphocyte populations in lymphoid tissues of ART+ animals showed evidence of restoration remains to be determined.

In contrast to our hypothesis, no overt signs of ART-associated toxicity were noted in CBA-administered animals. Our results show a modest yet significant increase in circulating creatinine levels following initiation of ART in both CBA/SIV and SUC/SIV animals. Small increases in plasma creatinine levels have been previously reported in HIV-infected patients treated with ART that includes emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) (Gallant et al., 2005; Cooper et al., 2010). The levels of creatinine detected during this period remained well within the normal range, and of limited biological significance. Our results suggest that at the doses used, the combination of FTC and tenofovir administered to SIV-infected macaques chronically exposed to daily CBA administration does not result in overt toxicity. Serial chemistries obtained throughout the study period revealed minor changes in several parameters including platelet count, hematocrit, cholesterol, and alkaline phosphatase levels between the sucrose and alcohol-treated animals. However, in each case changes were viewed minor in that values remained within the normal range for rhesus macaques.

Our results show a transient and significant increase in circulating levels of cholesterol early after SIV infection in CBA-administered macaques. Contrary to what we had predicted, no further alterations were noted during the ART period. Furthermore, no differences in serum triglyceride levels were observed in response to either CBA administration or SIV infection. Clinical studies have reported a high incidence of dyslipidemia and increased fat mass in alcoholics with more than 20% of patients meeting criteria for metabolic syndrome (Jarvis et al., 2007). Moreover, ART is associated with metabolic dysregulation characterized by dyslipidemia, insulin resistance, and lipodystrophy/lipoatrophy (Barbaro, 2006), and patients also present with altered adipokine profiles. Adipose tissue and circulating levels of adiponectin are lower in HIV-infected HAART patients (Kosmiski et al., 2003). An important factor that may explain the lack of overt changes in lipid profiles in our SIV/ART + animals is the dietary control and nutritionally balanced diet that the animals receive throughout the study. However, it is possible that additional more sensitive markers of altered lipid metabolism such as LDL, HDL, bile acids, acylcarnitines, sulfated steroids, and polyunsaturated fatty acids (PUFA) may provide further insight into the interaction of CBA with ART-associated metabolic alterations as shown in metabolomics studies (Casol, 2013). Parallel studies from our group are examining whether animals in this study show changes reflecting insulin resistance.

The ART formulation used in these studies (TDF/FTC) is widely prescribed as the backbone of combination ART in patients initiating treatment regimens, typically in combination with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) (Panel

on Antiretroviral Guidelines for Adults and Adolescents, 2014). This combination is also prescribed for pre-exposure prophylaxis (Center for Disease Control and Prevention, 2012, Center for Disease Control and Prevention, 2011). Previous studies have linked potential alcohol-toxicity to ART regimens that contained PI and NNRTI (Kumar, 2012) however this study limited evaluations to the NRTI backbone only. Additional studies are needed to evaluate efficacy of additional combination ART regimens. Our results do not support a significant interaction between alcohol and ART metabolism, however, it is possible that chronic alcohol consumption may affect metabolism of some antiretroviral drugs.. Chronic alcohol consumption has been shown to induce the expression and activity of CYP2B6 (Ferguson, 2012 and 2013), the principal enzyme involved in metabolism of the non-nucleoside reverse transcriptase inhibitor efavirenz. Reports from clinical studies indicate that alcohol consumption is a predictor of negative experiences with efavirenz-based ART (Sonenthal et al., 2014). Others have identified an association between alcohol use and HIV levels among those taking ART when controlling for adherence, thus, suggesting a potential drug interaction between ART and alcohol (Wu et al., 2011). In our studies with PMPA and FTC, we observed similar effectiveness in viral suppression in both the SUC and CBA-administered SIV-infected macaques, strongly suggesting that the overall kinetics of the drugs were not meaningfully altered. Additional studies with different ART regimens are warranted. Though we did not observe significant negative consequences in our studies, we strongly believe that reduction or cessation of alcohol consumption should be a goal for the management and care of all PLWHA.

The course of disease in CBA/SIV/ART- macaques did reflect small yet relevant differences in outcome measures including lower platelet count and %CD4 lymphocytes at the later times (3.5–4.5 MPSIV). Previous studies from our group have shown this stage of infection to be relatively asymptomatic and devoid of significant comorbid conditions like infections, diarrhea, and loss of body weight (Molina et al., 2006). Here we show that combination of CBA consumption with continuous NRTI-based ART in a controlled setting (optimal nutrition and no co-existing comorbid conditions) results in effective viral suppression in SIV-infected macaques. In summary, our study provides strong support for interventions aimed at either preventing or decreasing alcohol abuse in PLWHA. Furthermore, they provide support for continued counseling on relevance of continued ART therapy in individuals with AUD. These evidence-based findings will be used to promote adherence to ART in-care HIV+ patients.

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Figure 1.

Plasma viral load (mRNA log copies/ml) in sucrose (SUC)- and chronic binge alcohol (CBA)-administered macaques over time in months post-SIV infection, prior to- and after initiation of daily ART at 2.5 months post-SIV infection. Values are mean log viral loads $\pm$ SEM. Dotted lines and open symbols represent ART- and solid lines and filled symbols represent ART+ animals. Viral load (plasma mRNA) was analyzed as a log transformed variable (log-VL) with respect to all time points throughout the study period. No difference in mean response between the CBA- and SUC-administered groups was observed at any of the study time points. # $p < 0.05$ vs pre-ART (2.5 MPSIV).

Figure 2.

Circulating total lymphocyte count (A), % CD4 Lymphocytes (B), CD4 to CD8 ratio (C), and platelet count (D) in sucrose (SUC)- and chronic binge alcohol (CBA)-administered macaques over time in months post-SIV infection (MPSIV), prior to- and after initiation of uninterrupted ART at 2.5 MPSIV infection. Dotted lines and open symbols represent ART- and solid lines and filled symbols represent ART+ animals. # $p < 0.05$ vs pre-ART (2.5 MPSIV); * $p < 0.05$ vs. pre-SIV (0 MPSIV).

Figure 3.

Circulating total protein (A) and cholesterol (B) in sucrose (SUC)- and chronic binge alcohol (CBA)-administered macaques over time in months post-SIV infection, prior to- and after initiation of uninterrupted ART at 2.5 months post-SIV infection. Values are mean log viral loads $\pm$ SEM. Dotted lines and open symbols represent ART- and solid lines and filled symbols represent ART+ animals. N=6 per group. * $p < 0.05$ vs. pre-SIV (0 MPSIV); + $p < 0.05$ vs. time-matched treatment control.

Figure 4.

Circulating levels of Alkaline Phosphatase (A), Aspartate Aminotransferase (B), Creatinine (C), and Phosphorus (D) in sucrose (SUC)- and chronic binge alcohol (CBA)-administered macaques over time in months post-SIV infection, prior to- and after initiation of uninterrupted ART at 2.5 months post-SIV infection. Values are mean $\pm$ SEM. Dotted lines and open symbols represent ART- and solid lines and filled symbols represent ART+ animals. * $p < 0.05$ vs. pre-SIV; + $p < 0.05$ vs. time-matched treatment controls; # $p < 0.05$ vs pre-ART (2.5 MPSIV).

Table 1

Normal range for laboratory values for non-human primates.

Outcome measure	CBA	SUC	Laboratory Reference
Alanine Aminotransferase (U/L)	21 – 38	25 – 35	25 – 126
Albumin (g/dL)	3.4 – 4.0	3.3 – 4.0	3.0 – 5.9
Alkaline Phosphatase (U/L)	240 – 389	301 – 515	55 – 649
Aspartate Aminotransferase (U/L)	25 – 59	27 – 63	29 – 120
BUN (mg/dL)	9 – 18	14 – 18	13 – 27
Cholesterol (mg/dL)	121 – 148	110 – 126	106 – 241
Creatinine (U/L)	0.57 – 0.78	0.60 – 0.74	0.40 – 1.40
Gama-glutamyl Transferase (U/L)	39 – 54	46 – 54	32 – 89
Hematocrit (%)	34.6 – 38.3	36.5 – 39.5	34.8 – 55.2
Hemoglobin (g/dL)	10.7 – 12.1	11.4 – 12.3	10.1 – 55.2
Phosphorous (mg/dL)	4.2 – 5.8	4.0 – 6.2	3.4 – 7.5
Platelets ($\times 10^3/\mu\text{L}$)	394.2 – 501.1	351.9 – 500.4	193.1 – 676.2
Total Protein (g/dL)	6.4 – 6.9	6.2 – 7.1	5.9 – 7.8
Triglycerides	34 – 47	33 – 58	29 – 96