Published in final edited form as:

Expert Opin Drug Metab Toxicol. 2012 November ; 8(11): 1363–1375. doi:
10.1517/17425255.2012.714366.

Alcohol consumption effect on antiretroviral therapy and HIV-1 pathogenesis: role of cytochrome P450 isozymes

Santosh Kumar, PhD[†], Mengyao Jin, Anusha Ande, Namita Sinha, Peter S Silverstein, and Anil Kumar

University of Missouri Kansas City, School of Pharmacy, Kansas City, MO, USA

Abstract

Introduction—Alcohol consumption, which is highly prevalent in HIV-infected individuals, poses serious concerns in terms of rate of acquisition of HIV-1 infection, HIV-1 replication, response to highly active antiretroviral therapy (HAART) and AIDS/neuroAIDS progression. However, little is known about the mechanistic pathways by which alcohol exerts these effects, especially with respect to HIV-1 replication and the patient's response to HAART.

Areas covered—In this review, the authors discuss the effects of alcohol consumption on HIV-1 pathogenesis and its effect on HAART. They also describe the role of cytochrome P450 2E1 (CYP2E1) in alcohol-mediated oxidative stress and toxicity, and the role of CYP3A4 in the metabolism of drugs used in HAART (i.e., protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI)). Based on the most recent findings the authors discuss the role of CYP2E1 in alcohol-mediated oxidative stress in monocytes/macrophages and astrocytes, as well as the role of CYP3A4 in alcohol–PI interactions leading to altered metabolism of PI in these cells.

Expert opinion—The authors propose that alcohol and PI/NNRTI interact synergistically in monocytes/macrophages and astrocytes through the CYP pathway leading to an increase in oxidative stress and a decrease in response to HAART. Ultimately, this exacerbates HIV-1 pathogenesis and neuroAIDS.

Keywords

alcohol; antiretroviral; cytochrome P450; HIV-1; NeuroAIDS

1. Introduction

HIV-1 infection is a global problem with 34 million people affected in the world, of which, Sub-Saharan African countries account for > 20 million people [1]. Although the rate of acquisition of new HIV infections (2.7 million/year) and death due to AIDS-related causes (1.9 million/year) has been steady since 2007, there is an increase in the number of infected

© 2012 Informa UK, Ltd.

[†]Author for correspondence University of Missouri Kansas City, School of Pharmacy, 2464 Charlotte St., Kansas City, MO 64108, USA, Tel: +1 816 235 5494; Fax: +1 816 235 1776; kumarsa@umkc.edu.

Declaration of interest

The authors declare no other conflicts of interest.

people. One of the major challenges posed by HIV today is that the number of pregnant women in low-income countries who test positive for HIV-1 has dramatically increased from 8% in 2005 to 35% in 2010, leading to an unprecedented increase in HIV-infected children. However, nearly half of these patients, especially in countries in Sub-Saharan Africa, have no access to effective treatment to prevent mother–child transmission [1].

With the introduction of highly active antiretroviral therapy (HAART), there has been a substantial increase in the life expectancy of HIV-infected individuals [2-4]. However, the increased longevity of these individuals has resulted in an enhanced prevalence of neuroAIDS and HIV-associated neurodegenerative diseases (HAND), which poses a new challenge in the treatment of these populations [5-7]. HIV-associated dementia is the most severe form of HAND which is characterized by significantly reduced concentration and memory, as well as impairment of physical movements that compromise the lifestyle of HIV-1 patients [7]. HAND can cause neuropsychological impairments in as many as 60%, and neuropathological alterations in as many as 90%, of HIV-infected individuals [8]. An increase in neuropsychological impairments is consistent with an increased risk of consuming substances of abuse [9,10].

The prevalence of substance abuse, involving drugs such as alcohol, tobacco, methamphetamine or cocaine, is approximately threefold higher in HIV-infected patients than in the general population [10,11]. More importantly, individuals who are addicted to these substances rarely take one drug, and therefore the neurotoxic effects of consuming multiple drugs could be extremely severe [9,12]. The utilization of drugs of abuse is associated with the acquisition of HIV-1 infection, as well as increased rates of development of AIDS and neuroAIDS [13,14]. Methamphetamine and cocaine are known to increase the risk of HIV-1 infection through the use of shared needles for drug injection, as well as through an increase in unsafe sex practices [15,16]. Moreover, the abuse of these drugs in HIV-infected populations significantly reduces patient adherence to HAART regimens, which further exacerbates the neurological impairments caused by HIV-1 infection and neuroAIDS [17-20].

Several studies have demonstrated a role for inflammatory cytokines and chemokines in the interactions between HIV-1 and substances of abuse [21,22]. However, these pathways do not fully explain the interactions between drugs of abuse and antiretrovirals that have been observed, especially in the case of alcohol. Therefore, an alternate pathway, cytochrome P450 (CYP), has been proposed that could be involved not only in interactions between HIV-1 and drugs of abuse, but also in interactions between antiretrovirals and drugs of abuse [23], especially with alcohol. The author has also proposed that these interactions could lead to increased HIV-1 replication and decreased efficacy of HAART causing exacerbated HIV-1 pathogenesis and neuroAIDS. CYP is a superfamily of heme enzymes that are involved in the metabolism of a majority of therapeutic agents including protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI), as well as many substances of abuse such as alcohol and nicotine [24]. CYP enzymes do not metabolize or interact with other HAART drugs, such as nucleoside reverse transcriptase inhibitors (NRTI). In this review, we will discuss the possible role of the CYP pathways in alcohol-mediated effects on HIV-1 pathogenesis and HAART medications as follows: i) the effects

of alcohol on HIV-1 pathogenesis and neuroAIDS, ii) the role of the CYP pathway in alcohol-mediated oxidative stress and its implications in HIV-1 pathogenesis, iii) the role of the CYP system in alcohol–HAART interactions and its implications in HIV-1 pathogenesis and HAART treatment, iv) conclusions, and v) expert opinion on this subject. Figure 1 summarizes the target cells of HIV-1 infection, sites of ART action, sites of alcohol and PI/NNRTI metabolism, as well as cells or organs that are affected by HIV-1 pathogenesis and neuroAIDS.

2. Role of alcohol on HIV-1 pathogenesis and neuroAIDS

Heavy alcohol consumption and alcoholism are well-known to cause liver and pancreatic damage, as well as complications with various diseases [25,26]. Mild-to-moderate, as well as heavy drinking, is prevalent among HIV-infected individuals [27]. Although physicians recommend that HIV-infected patients, especially while on HAART treatment, abstain from alcohol, patient compliance is limited [19,28]. Current practice guidelines for HAART do not exclude patients who regularly consume mild-to-moderate levels of alcohol [19]. Chronic alcohol consumption during HAART is associated with decreased adherence to HAART [29], as well as increased neuronal toxicity [30]. Although moderate acute alcohol pre-exposure shows a protective effect on gp120-induced neurotoxicity in cell culture models [31], long-term moderate or heavy alcohol consumption exacerbates HIV pathogenesis, especially with neuroAIDS and results in decreased survival of HIV-infected patients [9,29,31-34].

Several reports using *in vitro* and *in vivo* studies have suggested that alcohol increases HIV-1 replication leading to enhanced immune suppression and more rapid progression to AIDS [35-40]. Alcohol is also known to disrupt the blood–brain barrier (BBB) and increase infiltration of HIV-1-infected monocytes/macrophages into the brain, where they can serve as a viral reservoir and spread viral infection to resident glia (i.e., perivascular macrophages, microglia, and astrocytes). This is one mechanism by which alcohol can exacerbate neuroinflammation and neuropsychological impairment [41-47]. Cells of the monocyte/macrophage lineage are one of the major cellular targets of HIV-1 and are relatively resistant to the cytopathic effect of HIV as these cells can survive infection with HIV for extended periods of time [42-44]. Although HAART has led to a significant increase in life expectancy [1-3,48,49], chronic alcohol consumption has been reported to decrease response to HAART [28,50].

Studies have shown that the antiviral activity of PI drugs, such as ritonavir and saquinavir, in chronically infected macrophages is approximately 2- to 10-fold lower than in chronically-infected lymphocytes [51,52]. However, the antiviral activity of NNRTI and NRTI is similar in both the cells. In order to have similar antiviral activity, relatively high concentration of these PIs is required in macrophages compared to lymphocytes. Therefore, strategies need to be developed to treat HIV-infected individuals, particularly strategies that target HIV-1 in chronically-infected macrophages [53,54]. Based on recent findings described in the following sections, it is likely that alcohol would further decrease the efficacy of PI in chronically-infected macrophages and lead to suboptimal treatment outcomes in alcohol consuming HIV-infected individuals. Thus, a determination of the pathway(s) involved in

alcohol–antiretroviral interactions, especially in HIV-infected macrophages, is important to find novel therapeutics and to optimize therapeutic regimens.

3. Role of cytochrome P450 pathway in alcohol-mediated oxidative stress and its implications with respect to HIV-1 pathogenesis

3.1 Alcohol and CYP2E1 in the liver

Cytochrome P450 pathways play a major role in the metabolic clearance of the majority of xenobiotics, including alcohol [24]. However, CYP2E1 plays a major role in alcohol-mediated damage to liver and other organs, as well as oral and liver cancers in chronic alcohol users [25,26,55-59]. These studies demonstrate that chronic ethanol consumption results in dramatically increased levels of CYP2E1 (up to 20-fold) leading to increased production of reactive oxygen species (ROS), lower cellular antioxidant levels, and enhanced oxidative stress. Although the alcohol dehydrogenase (ADH) is the main enzyme responsible for alcohol metabolism in the case of acute or social alcohol drinking, the highly alcohol-inducible CYP2E1 pathway plays a key role in oxidative stress-mediated liver damage caused by either binge drinking or chronic alcohol use. Recent findings suggest that certain polymorphisms of genes encoding CYP2E1 have altered metabolic activities and different associated risks for malignancies, such as oral and liver cancers, as a result of alcohol consumption [60]. Thus CYP2E1-mediated damage to liver or other organs has attracted attention in finding therapeutic interventions that target CYP2E1 and associated oxidative stress pathways to prevent liver injury as well as toxicity to other organs [61,62].

3.2 Alcohol and CYP2E1 in monocytes/macrophages

Reports from the literature have shown that several CYP enzymes, including CYP2E1, are expressed in monocyte/macrophage lineage of cells [63,64]. The amount of CYP2E1 relative to the total level of CYP isoforms (~ 3%) in a monocytic cell line (U937) [64] is similar to that of liver. In contrast, another alcohol-metabolizing enzyme, ADH, is expressed only at very low levels in these cells and is not thought to be responsible for alcohol metabolism in these cells [37,64]. The abundant expression of CYP2E1 in U937 cells and primary monocytes makes it important to investigate whether CYP2E1-mediated alcohol metabolism and the resultant oxidative stress are responsible for exacerbating HIV-1 infection in monocytes/macrophages. Our investigations have shown that acute alcohol treatment in U937 cells significantly increases the expression of CYP2E1, production of ROS, and induction of the antioxidant enzymes SOD1 and catalase [64]. Furthermore, oxidative stress generated through reactive oxygen and nitrogen species, as well as the imbalance in the levels of glutathione, has been shown to be associated with increased HIV-1 replication in monocytes/macrophages [65,66]. These findings further strengthen the hypothesis that CYP2E1 plays a key role in alcohol-mediated oxidative stress, and this may be a major mechanism responsible for the observed increase in HIV-1 replication in individuals who consume alcohol. It is of some relevance that alcohol has been demonstrated to increase the replication of hepatitis C virus through CYP2E1-mediated oxidative stress [67].

3.3 Alcohol and CYP2E1 in astrocytes

Recent reports from the literature have shown that several CYP enzymes are expressed in astrocytes [68,69]. CYP2E1 is expressed in the SVGA astrocytic cell line and primary astrocytes, and its relative expression (< 1%) compared to other CYP isozymes is lower compared to its expression in liver where CYP2E1 constitutes 4% of the total amount of CYP isoforms expressed. [69]. In contrast, ADH, another enzyme involved in alcohol metabolism, is not detectable in the brain [69]. Thus, the presence of CYP2E1 in astrocytes leads to the hypothesis that CYP2E1-mediated alcohol metabolism and the resultant oxidative stress could be involved in exacerbating the effects of HIV-1 on astrocytes.

3.4 Alcohol and CYP2E1 in neurons

Reports from the Haorah group suggest that the metabolism of ethanol in primary human neurons, along with ROS generation, is mainly mediated through CYP2E1, whereas the ADH activity is very low [48,70]. They have shown that a marked increase in a lipid peroxidation product (4-hydroxy-nonenal) and enhanced ROS generation coincides with decreased neuronal viability and diminished expression of neurofilament protein, a neuronal marker [70]. Exposure to alcohol also induces CYP2E1 which results in increased ROS generation, activation of myosin light chain (MLC) kinase, phosphorylation of MLC and TJ proteins, decreased BBB integrity, and enhanced monocyte migration across the BBB. Thus, oxidative stress resulting from alcohol metabolism in brain microvascular endothelial cells can lead to BBB breakdown in alcohol abuse and serve as an aggravating factor in neuroinflammatory disorders [48,70]. An *in vivo* study with rats and an *in vitro* study with human neuroblastoma IMR-32 cells in culture also suggest that CYP2E1 is induced in the brain by alcohol [71]. The induction of CYP2E1 may contribute to the CNS effects of alcohol and the development of nervous system pathologies observed in alcoholics [71]. Although, the role of CYP2E1 is well documented in alcohol-mediated BBB and neuronal damage, there is nothing known about the role of CYP2E1 in HIV-infected individuals who consume alcohol. Since both HIV-1 infection and alcohol are known to damage the BBB, it is likely that migration of HIV-infected monocytes/macrophages through the BBB is increased in HIV-infected alcoholics, leading to further neuronal damage. Therefore, it is imperative to study the CNS effects mediated by CYP2E1 in the context of HIV-1 infection/pathogenesis and the mechanism(s) by which CYP2E1 expression is regulated in the relevant cells.

3.5 Regulation of CYP2E1 expression in monocytes/macrophages and astrocytes by alcohol

The overall increase in the levels and/or enzymatic activity of CYP2E1 is controversial and is not well understood; many reports suggest that the expression levels and/or enzymatic activity of CYP2E1 are regulated at transcriptional and/or - post-translational levels [55-58]. For example, some studies suggest that CYP2E1 protein is stabilized by alcohol leading to increased levels of protein as well as enhanced enzymatic activity. Therefore, there is a critical need to examine the mechanism by which alcohol induces CYP2E1 in U937 monocytes/macrophages, as well as in astrocytes. Based on the evidence so far, it is suggested that chronic alcohol consumption increases oxidative stress through CYP2E1-

mediated alcohol metabolism, which further increases CYP2E1 induction in monocytes/macrophages and astrocytes [64,72]. An increase in CYP2E1 expression may further increase oxidative stress, which may eventually exacerbate HIV-1 pathogenesis in the CNS leading to enhanced progression of neuroAIDS in alcohol users (Figure 2).

3.6 Regulation of CYP2A6 expression in monocytes/macrophages by alcohol

In addition to a low level of alcohol metabolism [73], CYP2A6 is known to metabolize nicotine leading to the production of toxic metabolites and induction of oxidative stress, which in turn results in liver damage and lung cancer [74]. Since smoking is highly prevalent in alcoholic individuals (NIAAA and NIDA reports, 2011), the role of CYP2A6 in the context of alcohol and HIV-1 in monocytes/macrophages and astrocytes is of interest and clinical relevance [64,69,75,76]. CYP2A6 was the predominant CYP (~ 35%) present in monocytic cell lines U937 [64], and SVGA astrocytes [69]. Interestingly, alcohol increased the expression of CYP2A6 in both U937 monocytes and SVGA astrocytes, which led to an effort to determine the role of CYP2A6 in monocytes and SVGA astrocytes (Figure 2). The findings showed that CYP2A6 metabolizes nicotine into cotinine and other metabolites, leading to the generation of ROS in U937 monocytes and astrocytes [69,76]. This finding was confirmed by the use of tryptamine, a selective inhibitor of CYP2A6. Therefore, the mechanism by which alcohol regulates the expression of CYP2A6 was subsequently investigated [75]. The results showed that ethanol-mediated induction of CYP2A6 was completely blocked by both DAS and vitamin C, suggesting the role of CYP2E1-mediated oxidative stress in CYP2A6 induction. Furthermore, staurosporine, the inhibitor of PKC, and U0126, the inhibitor of mitogen-activated kinase kinase (MEK), completely abolished ethanol-induced CYP2A6 expression, while the activator of PKC (butylated hydroxyanisole) enhanced the expression of CYP2A6 [75]. The induction of CYP2A6 in the presence of these agents was consistent with the translocation of a nuclear factor, nuclear factor-related factor 2 (Nrf-2). Taken together, these results demonstrate that oxidative stress mediated by CYP2E1 activates the PKC/MEK/Nrf-2 pathway, leading to the expression of CYP2A6 in U937 monocytes (Figure 2).

4. Role of CYP3A4 in alcohol–HAART interaction and its implications with respect to HIV-1 pathogenesis

Since alcohol consumption is prevalent in HIV-infected individuals who are on HAART, there is a high risk of alcohol–ART interactions mediated by CYP3A4, primarily because all the PI and NNRTI (two major components of HAART regimens) are substrates, and some of them are inhibitors and inducers, of CYP3A4 [77]. These interactions may lead to altered response to HAART regimens and increased toxicity, which in turn may be responsible for increased HIV-1 replication, especially in monocytes/macrophages.

4.1 CYP3A4 in the liver

Despite the success of HAART, it has created serious therapeutic challenges through drug–drug interaction (DDI) as a result of induction (mRNA and protein levels) or inhibition (enzymatic activity) of CYP3A4 by many PI and NNRTI [77-79]. Use of a CYP3A4 inhibitor can prolong the elimination of drug substrates leading to increased half-life and

toxic drug accumulation in the liver [80]. Alternatively, a CYP3A4 inducer may lead to suboptimal drug concentrations and a reduced therapeutic effect of HAART drugs due to faster metabolism [81]. All PI and NNRTI are substrates of CYP3A4, while efavirenz is also a substrate of CYP2B6. While some NNRTI are both inducers and inhibitors of CYP3A4, all PI are inhibitors of CYP3A4. Ritonavir is the strongest inhibitor, as well as an inducer [77-80] of CYP3A4 in the liver. However, because of its strong binding affinity to CYP3A4 and effective inhibition of CYP3A4, it is generally used as a booster to increase the plasma concentrations of other PI. Alcohol has been shown to induce the expression of CYP3A4 in the liver, and this may lead to relatively faster metabolism of many drugs, especially PI and NNRTI and reduced efficacy of these antiretrovirals [82]. However, the mechanism by which alcohol induces CYP3A4 expression is not clear.

4.2 CYP3A4 in monocytes/macrophages, astrocyte, and neurons

CYP3A4 has also been demonstrated to be expressed in BBB endothelial cells and in neurons [83]. CYP3A4 brain expression is not only associated with drug metabolism but may also be involved in a cytoprotective mechanism, because CYP3A4 expression is inversely correlated with DAPI nuclear condensation and positively correlated with the amount of carbamazepine metabolism. Accumulation of carbamazepine is known to cause cell toxicity. Further, a recent study has shown that CYP3A4 is expressed in monocytes/macrophages [64]. Since, for maximal effect, PI must act in HIV-infected macrophages as well as in T cells, and the EC_{50} of PI is higher in chronically infected macrophages than in T lymphocytes [51-53], a determination of the role of CYP3A4 in the metabolism and bioavailability of PI in monocytes/macrophages is important. The low efficacy of PI in monocytes/macrophages could be a result of relatively higher levels of CYP3A4 in these cells compared to lymphocytes. In this line of investigation, it has recently been shown that CYP3A4 is the predominant CYP isoform expressed in U937 monocytes/macrophages [64]. Alcohol significantly increases the expression of CYP3A4 in U937 monocytes/macrophages [64], which is further expected to decrease the bioavailability of PI in monocytes/macrophages leading to additional decreases in antiviral activity in these cells. Although the presence of CYP3A4 is documented in monocytes/macrophages, astrocytes, BBB, and neurons, there is very little known in terms of metabolism of PI or NNRTI in the context of both HIV-1 infection and alcohol in these cells.

4.3 Alcohol–CYP3A4–PI interactions

Although CYP3A4 induction by alcohol and the impact of CYP3A4 on drug metabolism and toxicity is well-known, CYP3A4–ethanol physical interactions and their impact on drug binding, inhibition, or metabolism remains unknown. Therefore, all currently prescribed PI that differ in physicochemical properties were used in a study to determine the effects of ethanol on CYP3A4–PI binding [84,85]. The initial result showed that alcohol alone binds the CYP3A4 active site using a type I binding characteristic, i.e., non-covalent binding of alcohol with the heme-Fe of CYP3A4 by replacement of a water molecule and characterized by a relatively strong binding affinity ($K_D = 6 \pm 0.34$ mM) [84]. Furthermore, atazanavir, lopinavir, saquinavir, and tipranavir showed type I spectral binding, whereas indinavir and ritonavir showed type II binding [86]. Type II binding occurs through covalent interaction between the heme-Fe of CYP3A4 and ligands. However, amprenavir and darunavir did not

exhibit spectral binding with CYP3A4. Ethanol at 20 mM (0.1%) decreased the maximum spectral change ($\Delta A_{\max}$) with the type I PIs lopinavir and saquinavir, but it did not alter $\Delta A_{\max}$ with other PIs. Ethanol did not alter the spectral binding affinity (K_D) or the inhibition constant (IC_{50}) of type I PI. However, ethanol significantly decreased the IC_{50} of type II PI, indinavir and ritonavir, and markedly increased the IC_{50} of amprenavir and darunavir.

This study suggests a differential effect of ethanol on spectral binding and inhibitory characteristics of type I (atazanavir, lopinavir, saquinavir, and tipranavir), type II (indinavir and ritonavir), and spectrally unbound (amprenavir and darunavir) PI. The lack of change in the binding affinity of type I PI in the presence of alcohol suggests that alcohol is not expected to alter the metabolism and therefore efficacy of type I PI. However, alcohol is expected to alter the metabolism and efficacy of type II PI and the PI that does not show spectral change (Figure 3). The altered binding and inhibition of CYP3A4 with these PI may have implications on their metabolism and efficacy in alcoholic HIV-1-infected individuals. A decrease in the magnitude of spectral binding with lopinavir and saquinavir and an increase in the binding affinity by the CYP3A4 inhibitors indinavir and ritonavir may alter the metabolism of these drugs by CYP3A4. The altered inhibition of CYP3A4 by these PI may also affect the metabolism of these PI, as well as other PI or NNRTI. However, it is difficult to predict whether an increased binding affinity or inhibition by a PI will increase or decrease the metabolism of the PI because the site of interaction of a PI with the CYP3A4 heme-Fe for binding/inhibition and metabolism could be different. An increased metabolism is likely to decrease the efficacy of PI, whereas a decreased metabolism may increase the efficacy of PI. In either case, however, the toxicity is likely to increase as a result of accumulation of either drugs or drug metabolites. Therefore, metabolic studies of these PI, as well as NNRTI in the presence of alcohol are necessary. Nonetheless, this is an important finding in context with the report that alcohol is known to increase the toxicity and reduce the efficacy of HAART in HIV-1-infected individuals [26,28,86-88]. More specifically, the studies from Flexner and colleagues [88] and Shibata and colleagues [89,90] have shown that alcohol can increase the metabolism and decrease the bioavailability of PI and NNRTI, resulting in decreased efficacy. The presence of multiple PI and/or NNRTI in HAART regimen, in which PI also act as CYP3A4 inhibitors, make alcohol-CYP3A4-PI interactions complex. Therefore, this study provides the first step toward understanding the overall effect of ethanol on the metabolism, bioavailability, and efficacy of PI and NNRTI in HIV-1-infected individuals who are chronic mild-to-moderate alcohol users.

5. Conclusions

The impact of substance abuse, especially alcohol consumption, on various aspects of HIV pathogenesis and treatment is well documented in the literature. Therefore, there is a critical need to address this issue in order to minimize these effects in HIV+ patients who engage in either mild-to-moderate or heavy alcohol consumption. Although the role of inflammatory cytokines in these cases has been demonstrated, there is a critical need to investigate alternate pathways, such as cytochrome P450 that may be responsible for alcohol-mediated effects on HIV-1 pathogenesis and the response to HAART drugs. The available evidence suggests that CYP2E1 plays an important role in alcohol-mediated oxidative stress in

monocytes/macrophages and astrocytes, and this may result in increased HIV-1 replication and toxicity in these cells. The results described in this report suggest that CYP3A4 plays an important role in alcohol–PI interactions leading to altered metabolism of PI and NNRTI, thereby increasing drug-mediated toxicity and decreasing efficacy of these drugs. Both CYP2E1- and CYP3A4-mediated effects by alcohol may ultimately increase HIV-1 replication and thus decrease the response to HAART in HIV-infected individuals who chronically consume alcohol. Thus, there is an urgent need to further examine the contribution of the CYP pathway in alcohol-mediated effects on HIV-1 pathogenesis using both *in vitro* and *in vivo* approaches (clinical samples) as described in the expert opinion section. This would provide strong scientific evidence to support the avoidance or elimination of alcohol use for HIV-infected individuals, especially while they are on HAART. Since it is difficult for the HIV patients to comply, this would promote the discovery of novel interventions, as well as determine optimal drug dose adjustments for HIV-infected alcohol users and improve their overall health and quality of life.

6. Expert opinion

The PIs used in the HAART regimen are expected to interact with alcohol thereby altering their metabolism and antiviral activity, especially in HIV-infected monocytes/macrophages [82,87,89,90]. Thus, HIV-infected individuals who consume alcohol and take PIs are not only affected by decreased drug efficacy, but are also at high risk for deleterious alcohol–PI interactions leading to drug toxicity. Since PIs show lower levels of antiviral activity in chronically-infected macrophages compared to lymphocytes, they have been replaced in some instances with integrase inhibitors which have shown a relatively high antiviral activity in macrophages, quite possibly because integrase inhibitors are not metabolized by CYP3A4 [91]. We propose that alcohol-induced CYP3A4 is likely to increase the metabolism of PIs and decrease their bioavailability, and this may be partially responsible for a poor patient response to antiretroviral drugs (Figure 4). In addition, PI and NNRTI are also known to induce CYP3A4, and this is likely to increase the metabolism of PI and NNRTI and also decrease the response to HAART. We propose that alcohol–PI/NNRTI interactions will synergistically increase CYP expression; this may lead to a further decrease in response to HAART and an increase in toxicity mediated by drug metabolites (Figure 4). On the other hand, PIs which are inhibitors of CYP3A4 may decrease the metabolism of antiretrovirals and thereby decrease the clearance of PI and NNRTI. Thus, these CYP3A4 inhibitors are likely to increase the accumulation of other PI and NNRTI leading to potentially increased efficacy, but significantly increased drug toxicity as well. Alcohol and PI (CYP3A4 inhibitor) are also likely to interact with CYP3A4 physically causing complex three-way alcohol–CYP3A4–PI interactions which result in altered metabolism of NNRTI and PI in the liver as well as monocytes/macrophages [84,85]. However, the final outcome of these complex interactions is difficult to predict.

Therefore, an extensive study using treatments with alcohol and different clinically relevant combinations of PI/NNRTI in HIV-infected macrophages and astrocytes, as well as in hepatocytes and neurons, needs to be undertaken. This will help define the fate of these drugs along with any associated effects on drug efficacy and toxicity in these cells. Furthermore, potential *in vivo* alcohol–PI/NNRTI interactions can be determined by

determining *in vitro* CYP3A4 activity in the absence and presence of alcohol and PI/NNRTI using recombinant CYP3A4. The *predicted in vivo* CYP3A4 activity can be calculated by estimating the K_i of PI/NNRTI for CYP3A4 and using literature-derived values of $C_{\max}$ for inhibitor and substrate (PI/NNRTI) as described previously [92]. Then, “*predicted in vivo*” concentrations of NNRTI and PI can be determined as described in the literature [93,94]. We hypothesize that ethanol and PI/NNRTI together will show additive or synergistic effects on the induction of CYP3A4 in these cells, which may significantly decrease the levels of PI and NNRTI. This may significantly decrease the antiretroviral activity of PI and NNRTI in HIV-infected cells in the periphery, as well as in perivascular macrophages, microglia, and astrocytes. The data from alcohol-PI physical interactions will provide *predicted in vivo* concentrations of PI and NNRTI. A final model based on the inductive and inhibitory effects of alcohol in the presence of each PI/NNRTI regimen can be obtained, and this is expected to demonstrate the efficacy of each regimen in the presence of alcohol and toxicity mediated by these regimens.

Ritonavir, which is the strongest inhibitor of CYP3A4, is prescribed along with most of the PIs to boost their concentrations [95,96]. Although, saquinavir, nelfinavir, and indinavir are the oldest and best-characterized PIs, currently lopinavir/ritonavir, darunavir/ritonavir, and atazanavir/ritonavir are commonly prescribed PIs in HAART regimens [97]. Lopinavir/ritonavir and darunavir/ritonavir combinations are increasingly prescribed for HIV-1 patients and these combinations also have the capability to cross the BBB and treat HIV-1 infection in the brain. Of these combinations, darunavir/ritonavir is the first choice for HAART regimens because this PI combination is the most cost-effective [98]. Therefore, it is essential to study the implications of alcohol in CYP3A4-mediated metabolism of these, as well as other PI, in monocytes/macrophages, astrocytes, BBB, and neurons.

The results of *in vitro* studies, however, need to be complemented with *ex vivo* cross-sectional and/or *in vivo* longitudinal studies using alcoholic, HIV-infected, and HIV-infected alcoholic individuals, especially using monocytes and macrophages. Based on *in vitro* and *in vivo* studies, a decrease in the efficacy of PI/NNRTI and an increase in alcohol- and PI/NNRTI-mediated toxicity are likely to increase HIV-1 replication in monocytes and macrophages, which would ultimately increase HIV-1 pathogenesis and exacerbate the development of neuroAIDS and the prevalence of HAND. These studies would test the hypothesis that alcohol enhances HIV-1 replication through CYP-mediated oxidative stress in human macrophages. This would also test the hypothesis that alcohol reduces the efficacy of PI/NNRTI through the CYP3A4-mediated pathway.

The successful completion of this study would provide strong evidence in support of avoidance of alcohol use for HIV+ patients, especially while they are on HAART. In addition, it would also provide an opportunity to develop novel therapeutic agents that effectively treat HIV+ patients who use alcohol by targeting the regulatory pathways involved in the expression of CYP2E1 and CYP3A4. CYP pathways are important targets for developing novel drugs for many diseases, including several types of cancer [61,99]. In addition, selective antioxidants can be employed in new strategies to combat alcohol-mediated effects in HIV-infected individuals [62]. This study could also be expanded to examine the effects of co-infection, such as TB and hepatitis, which are common infections

among HIV-infected individuals [100]. Simultaneous treatment of these co-infections using multiple drugs further increases the risk of DDI and adverse drug reactions [101]. For example, rifampicin, the major drug that is used to treat TB is a strong inducer of CYP3A4, which is likely to increase the metabolism of PI/NNRTI and thereby decrease their efficacy. Therefore, it is pertinent to optimize the dose of each drug in common drug regimens while treating for both TB and HIV-1 infections simultaneously. Finally, further studies can be undertaken to examine the effects of other drugs that are commonly abused in conjunction with alcohol, such as tobacco and methamphetamine. These substances are likely to also have an effect on HIV-1 replication and efficacy of HAART [22,23,102].

Acknowledgments

Financial support was provided by NIH Grants DA031616 and AA020806.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Global HIV/AIDS Response. Epidemic update and health sector progress towards Universal Access – Progress Report. World Health Organization; 2011. p. 11-47.
- 2••. Burgoyne RW, Tan DHS. Prolongation and quality of life for HIV-infected adults treated with highly active antiretroviral therapy (HAART): a balancing act. *J Antimicrob Chemother.* 2008; 61:469–73. Introduction of HAART significantly improved the quality of life and life expectancy in HIV-infected individuals. [PubMed: 18174196]
3. Hogg RS, Heath KV, Yip B, et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *J Am Med Ass.* 1998; 279:450–4.
4. May MT, Ingle SM. Life expectancy of HIV-positive adults: a review. *Sex Health.* 2011; 8:526–33. [PubMed: 22127039]
- 5•. Mothobi NZ, Brew BJ. Neurocognitive dysfunction in the highly active antiretroviral therapy era. *Curr Opin Infect Dis.* 2012; 25:4–9. The prevalence of HAND remains high in the HAART era and additional studies are needed to reduce the prevalence and severity of HAND in HIV-infected individuals. [PubMed: 22156897]
6. Vivithanaporn P, Gill MJ, Power C. Impact of current antiretroviral therapies on neuroAIDS. *Expert Rev Anti Infect Ther.* 2011; 9:371–4. [PubMed: 21504390]
7. Xia C, Luo D, Yu X, et al. HIV-associated dementia in the era of highly active antiretroviral therapy (HAART). *Microbes Infect.* 2011; 13:419–25. [PubMed: 21262373]
8. Boissé L, Gill MJ, Power C. HIV infection of the central nervous system: clinical features and neuropathogenesis. *Neurol Clin.* 2008; 26:799–819. [PubMed: 18657727]
9. Lucas S. Causes of death in the HAART era. *Curr Opin Infect Dis.* 2012; 25:36–41. [PubMed: 22156899]
- 10••. Purohit V, Rapaka R, Shurtleff D. Drugs of abuse, dopamine, and HIV-associated neurocognitive disorders/HIV-associated dementia. *Mol Neurobiol.* 2011; 44:102–10. Drugs of abuse, especially cocaine and methamphetamine, exacerbate HIV-1 pathogenesis, neuroAIDS, and HIV-associated dementia. [PubMed: 21717292]
11. Chander G. Addressing alcohol use in HIV-infected persons. *Top Antivir Med.* 2011; 19:143–7. [PubMed: 22156216]
12. Mehta SH, Lucas G, Astemborski J, et al. Early immunologic and virologic responses to highly active antiretroviral therapy and subsequent disease progression among HIV-infected injection drug users. *AIDS Care.* 2007; 19:637–45. [PubMed: 17505924]

13. Chander G, Himelhoch S, Fleishman JA, et al. HAART receipt and viral suppression among HIV-infected patients with co-occurring mental illness and illicit drug use. *AIDS Care*. 2009; 21:655–63. [PubMed: 19444675]
- 14••. Norman LR, Kumar A. Neuropsychological complications for HIV disease and substance of abuse. *Am J Infect Dis*. 2006; 2:115–24. Psychiatric risk factors, particularly substance use, which often contribute to initial acquisition of HIV, also exacerbate neuropsychological dysfunction.
15. Booth RE, Watters JK, Chitwood DD. HIV risk-related sex behaviors among injection drug users, crack smokers, and injection drug users who smoke crack. *Am J Public Health*. 1993; 83:1144–8. [PubMed: 8342724]
16. Hatfield LA, Horvath KJ, Jacoby SM, Simon BR. Comparison of substance use and risky sexual behavior among a diverse sample of urban, HIV-positive men who have sex with men. *J Addict Dis*. 2009; 28:208–18. [PubMed: 20155589]
- 17•. Gonzalez A, Barinas J, O’Cleirigh C. Substance use: impact on adherence and HIV medical treatment. *Curr HIV/AIDS rep*. 2011; 8:223–34. Substance use is highly prevalent among people living with HIV/AIDS, which significantly reduces the adherence to HAART treatment. [PubMed: 21858414]
- 18•. Chander G, Lau B, Moore R. Hazardous alcohol use: a risk factor for non-adherence and lack of suppression in HIV infection. *J Ac Imm Def Syn*. 2006; 43:411. Hazardous alcohol use alone and in combination with injection drug use are associated with decreased HAART uptake, adherence, and viral suppression.
19. Hirsch MS, Günthard HF, Schapiro JM, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 Recommendations of an International AIDS Society–USA Panel. *Clin Infect Dis*. 2008; 47:266–85. [PubMed: 18549313]
20. Witteveen E, van Ameijden EJ. Drug users and HIV-combination therapy (HAART): factors which impede or facilitate adherence. *Subst Use Misuse*. 2002; 37:1905–25. [PubMed: 12511058]
21. Cabral GA. Drugs of abuse, immune modulation, and AIDS. *J Neuroimmune Pharmacol*. 2006; 1:280–95. [PubMed: 18040805]
22. Silverstein PS, Shah A, Gupte R, et al. Methamphetamine toxicity and its implications during HIV-1 infection. *J Neurovirol*. 2011; 17:401–15. [PubMed: 21786077]
- 23••. Kumar S. Role of cytochrome P450 systems in substance of abuse mediated HIV-1. Pathogenesis and NeuroAIDS. *J Drug Metab Toxicol*. 2012; 2:1–3. Cytochrome P450 is proposed to play important role in substance of abuse, especially alcohol and tobacco-mediated HIV-1 pathogenesis and response to HAART drugs.
- 24•. Anzenbacher P, Anzenbacherová E. Cytochromes P450 and metabolism of xenobiotics. *Cell Mol Life Sci*. 2001; 58:737–47. Cytochrome P450 is the major enzyme system that is involved in the metabolic clearance of 80% of the marketed drugs and many other xenobiotics, including alcohol. [PubMed: 11437235]
- 25••. Clemens DL, Mahan KJ. Alcoholic pancreatitis: lessons from the liver. *World J Gastroenterol*. 2010; 16:1314–20. CYP2E1 is the major enzyme involved in alcohol-mediated oxidative stress leading to liver damage and pancreatitis in chronic alcohol users. [PubMed: 20238397]
26. Mellion M, Gilchrist JM, de la Monte S. Alcohol-related peripheral neuropathy: nutritional, toxic, or both? *Muscle Nerve*. 2011; 43:309–16. [PubMed: 21321947]
27. Galvan FH, Bing EG, Fleishman JA, et al. The prevalence of alcohol consumption and heavy drinking among people with HIV in the United States: results from the HIV Cost and Services Utilization Study. *J Stud Alcohol*. 2002; 63:179–86. [PubMed: 12033694]
28. Miguez MJ, Shor-Posner G, Morales G, et al. HIV treatment in drug abusers: impact of alcohol use. *Addict Biol*. 2003; 8:33–7. [PubMed: 12745413]
29. Hendershot CS, Stoner SA, Pantalone DW, Simoni JM. Alcohol use and antiretroviral adherence: review and meta-analysis. *J Acquir Immune Defic Syndr*. 2009; 52:180–202. [PubMed: 19668086]
30. Ferrari LF, Levine JD. Alcohol consumption enhances antiretroviral painful peripheral neuropathy by mitochondrial mechanisms. *Eur J Neurosci*. 2010; 32:811–18. [PubMed: 20726883]
31. Belmadani A, Neafsey EJ, Collins MA. Human immunodeficiency virus type 1 gp120 and ethanol coexposure in rat organotypic brain slice cultures: curtailment of gp120-induced neurotoxicity and

- neurotoxic mediators by moderate but not high ethanol concentrations. *J Neurovirol.* 2003; 9:45–54. [PubMed: 12587068]
32. Shacham E, Agbebi A, Stamm K, Overton ET. Alcohol consumption is associated with poor health in HIV clinic patient population: a behavioral surveillance study. *AIDS Behav.* 2011; 15:209–13. [PubMed: 20013042]
 33. Acheampong E, Mukhtar M, Parveen Z, et al. Ethanol strongly potentiates apoptosis induced by HIV-1 proteins in primary human brain microvascular endothelial cells. *Virology.* 2002; 304:222–34. [PubMed: 12504564]
 34. Persidsky Y, Ho W, Ramirez SH, et al. HIV-1 infection and alcohol abuse: neurocognitive impairment, mechanisms of neurodegeneration and therapeutic interventions. *Brain Behav Immun.* 2011; 25:61–70. Oxidative stress, overproduction of pro-inflammatory factors, impairment of blood–brain barrier and glutamate-associated neurotoxicity play important roles in alcohol and HIV-1-mediated neurodegeneration.
 35. Bagasra O, Bachman SE, Jew L, et al. Increased human immunodeficiency virus type 1 replication in human peripheral blood mononuclear cells induced by ethanol: potential immunopathogenic mechanisms. *J Infect Dis.* 1996; 173:550–8. Ethanol-infused subjects' peripheral blood mononuclear cells exhibit significantly increased replication of HIV-1, which is associated with increased inhibition of CD8⁺ T lymphocytes' function by alcohol. [PubMed: 8627016]
 36. Bagasra O, Kajdacsy-Balla A, Lischner HW, Pomerantz RJ. Alcohol intake increases human immunodeficiency virus type 1 replication in human peripheral blood mononuclear cells. *J Infect Dis.* 1993; 167:789–97. [PubMed: 8450242]
 37. Haorah J, Heilman D, Diekmann C, et al. Alcohol and HIV decrease proteasome and immunoproteasome function in macrophages: implications for impaired immune function during disease. *Cell Immunol.* 2004; 229:139–48. [PubMed: 15474528]
 38. Kumar R, Perez-Casanova AE, Tirado G, et al. Increased viral replication in simian immunodeficiency virus/simian-HIV-infected macaques with self-administering model of chronic alcohol consumption. *J Acquir Immune Defic Syndr.* 2005; 39:386–90. Alcohol consumption increases HIV-1 replication and viral loads in SIV/HIV-infected macaques, suggesting the role of alcohol in HIV-1 replication. [PubMed: 16010157]
 39. Marcondes MC, Watry D, Zandonatti M, et al. Chronic alcohol consumption generates a vulnerable immune environment during early SIV infection in rhesus macaques. *Alcohol Clin Exp Res.* 2008; 32:1583–92. [PubMed: 18616669]
 40. Baum MK, Rafie C, Lai S, et al. Alcohol use accelerates HIV disease progression. *AIDS Res Hum Retroviruses.* 2010; 26:511–18. Frequent alcohol intake and the combination of frequent alcohol and crack-cocaine accelerate HIV disease progression. [PubMed: 20455765]
 41. Buckner CM, Luers AJ, Calderon TM, et al. Neuroimmunity and the blood-brain barrier: molecular regulation of leukocyte transmigration and viral entry into the nervous system with a focus on neuroAIDS. *J Neuroimmune Pharmacol.* 2006; 1:160–81. [PubMed: 18040782]
 42. Kedzierska K, Crowe SM. The role of monocytes and macrophages in the pathogenesis of HIV-1 infection. *Curr Med Chem.* 2002; 9:1893–903. [PubMed: 12369874]
 43. Cobos-Jiménez V, Booiman T, Hamann J, Kootstra NA. Macrophages and HIV-1. *Curr Opin HIV AIDS.* 2011; 6:385–90. Monocytes/macrophages are one of targets of HIV-1 infection and major viral reservoirs, which infiltrate into the CNS and infect perivascular macrophages and microglia. [PubMed: 21734564]
 44. Kramer-Hämmerle S, Rothenaigner I, Wolff H, et al. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res.* 2005; 111:194–213. [PubMed: 15885841]
 45. Pfefferbaum A, Rosenbloom MJ, Rohlfing T, et al. Contribution of alcoholism to brain dysmorphology in HIV infection: effects on the ventricles and corpus callosum. *Neuroimage.* 2006; 33:239–51. Alcohol consumption in HIV-infected individuals leads to neurotoxicity thereby causing altered morphology of brain, especially in the ventricles and corpus callosum. [PubMed: 16877010]
 46. Pfefferbaum A, Rosenbloom MJ, Sassoon SA, et al. Regional brain structural dysmorphology in human immunodeficiency virus infection: effects of acquired immune deficiency syndrome,

- alcoholism, and age. *Biol Psychiatry*. 2012 Published online march 26, 2012. 10.1016/j.biopsych.2012.02.018
47. Rothlind JC, Greenfield TM, Bruce AV, et al. Heavy alcohol consumption in individuals with HIV infection: effects on neuropsychological performance. *J Int Neuropsychol Soc*. 2005; 11:70–83. [PubMed: 15686610]
 48. Haorah J, Knipe B, Leibhart J, et al. Alcohol-induced oxidative stress in brain endothelial cells causes blood-brain barrier dysfunction. *J Leukoc Biol*. 2005; 78:1223–32. Oxidative stress resulting from alcohol metabolism can lead to blood–brain barrier breakdown in alcohol abuse, which serves as an aggravating factor in neuroinflammatory disorders. [PubMed: 16204625]
 49. Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. *Antiviral Res*. 2010; 85:1–18. [PubMed: 20018391]
 50. Neblett RC, Hutton HE, Lau B, et al. Alcohol consumption among HIV-infected women: impact on time to antiretroviral therapy and survival. *J Womens Health (Larchmt)*. 2011; 20:279–86. [PubMed: 21281111]
 51. Perno CF, Newcomb FM, Davis DA, et al. Relative potency of protease inhibitors in monocytes/macrophages acutely and chronically infected with human immunodeficiency virus. *J Infect Dis*. 1998; 178:413–22. The potency of protease inhibitors in HIV-infected monocytes/macrophages is much lower than lymphocytes, thereby decreasing the antiviral activity of protease inhibitors in monocytes/macrophages compared to lymphocytes. [PubMed: 9697721]
 52. Perno CF, Balestra E, Francesconi M, et al. Antiviral profile of HIV inhibitors in macrophages: implications for therapy. *Curr Top Med Chem*. 2004; 4:1009–15. [PubMed: 15134554]
 53. Aquaro S, Svicher V, Schols D, et al. Mechanisms underlying activity of antiretroviral drugs in HIV-1-infected macrophages: new therapeutic strategies. *J Leukoc Biol*. 2006; 80:1103–10. [PubMed: 16931601]
 54. Gavegnano C, Schinazi RF. Antiretroviral therapy in macrophages: implication for HIV eradication. *Antivir Chem Chemother*. 2009; 20:63–78. [PubMed: 19843977]
 55. Gonzalez FJ. The 2006 Bernard B. Brodie award lecture: CYP2E1. *Drug Metab Dispos*. 2007; 35:1–8. CYP2E1 is mainly involved in alcohol-mediated oxidative stress leading to liver damage and damage of other organs in chronic alcohol users. [PubMed: 17020953]
 56. Millonig G, Wang Y, Homann N, et al. **Ethanol**-mediated carcinogenesis in the human esophagus implicates **CYP2E1** induction and the generation of carcinogenic DNA-lesions. *Int J Cancer*. 2011; 128:533–40. [PubMed: 20715111]
 57. Jing L, Jin CM, Li SS, et al. Chronic alcohol intake-induced oxidative stress and apoptosis: role of CYP2E1 and calpain-1 in alcoholic cardiomyopathy. *Mol Cell Biochem*. 2012; 359:283–92. [PubMed: 21833537]
 58. Tang H, Sebastian BM, Axhemi A, et al. Ethanol-induced oxidative stress via the CYP2E1 pathway disrupts adiponectin secretion from adipocytes. *Alcohol Clin Exp Res*. 2012; 36:214–22. [PubMed: 21895711]
 59. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. *Arch toxicol*. 2009; 83:519–48. CYP2E1-mediated oxidative stress plays major role in liver toxicity in chronic alcohol users. [PubMed: 19448996]
 60. Marichalar-Mendia X, Rodriguez-Tojo MJ, Acha-Sagredo A, et al. Oral cancer and polymorphism of ethanol metabolising genes. *Oral Oncol*. 2010; 46:9–13. [PubMed: 19896412]
 61. Karamanakos PN, Trafalis DT, Geromichalos GD, et al. Inhibition of rat hepatic CYP2E1 by quinacrine: molecular modeling investigation and effects on 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced mutagenicity. *Hum Retroviruses*. 2006; 22:589–94.
 62. Bishayee A, Darvesh AS, Politis T, McGory R. Resveratrol and liver disease: from bench to bedside and community. *Liver Int*. 2010; 8:1103–14. [PubMed: 20557453]
 63. Hutson JL, Wickramasinghe SN. Expression of CYP2E1 by human monocyte-derived macrophages. *J Pathol*. 1999; 188:197–200. [PubMed: 10398164]
 64. Jin M, Arya P, Patel K, et al. Effect of alcohol on drug efflux protein and drug metabolic enzymes in U937 macrophages. *Alcohol Clin Exp Res*. 2011; 35:132–9. Several cytochrome P450 enzymes, including CYP2E1 and CYP3A4, are substantially expressed in U937 monocyte-derived macrophages and these enzymes are induced by alcohol. [PubMed: 21039635]

65. Aquaro S, Muscoli C, Ranazzi A, et al. The contribution of peroxynitrite generation in HIV replication in human primary macrophages. *Retrovirology*. 2007; 4:74–6. [PubMed: 17937800]
66. Mialocq P, Oiry J, Puy JY, et al. Oxidative metabolism of HIV-infected macrophages: the role of glutathione and a pharmacologic approach. *Pathol Biol (Paris)*. 2001; 49:567–71. [PubMed: 11642020]
67. McCartney EM, Beard MR. Impact of alcohol on hepatitis C virus replication and interferon signaling. *World J Gastroenterol*. 2010; 16:1337–43. Alcohol causes replication of hepatitis C virus through CYP2E1-mediated oxidative stress. [PubMed: 20238400]
68. Malaplate-Armand C, Leininger-Muller B, Batt AM. Astrocytic cytochromes p450: an enzyme subfamily critical for brain metabolism and neuroprotection. *Rev Neurol (Paris)*. 2004; 160:651–8. Cytochromes P450 enzymes are expressed in astrocytes and play a role in brain metabolism of drugs and other xenobiotics, and in neuroprotection. [PubMed: 15247853]
69. Ande A, Earla R, Jin M, et al. An LC-MS/MS method for concurrent determination of nicotine metabolites and the role of CYP2A6 in nicotine metabolite-mediated oxidative stress in SVGA astrocytes. *Drug Alcohol Depend*. 2012 published online April 10, 2012. 10.1016/j.drugalcdep.2012.03.015
70. Haorah J, Ramirez SH, Floreani N, et al. Mechanism of alcohol-induced oxidative stress and neuronal injury. *Free Radic Biol Med*. 2008; 45:1542–50. Metabolism of ethanol in primary human neurons by ADH or CYP2E1 generates reactive oxygen species and nitric oxide, leading to increase in the lipid peroxidation, decreased neuronal viability, and diminished expression of neuronal marker. [PubMed: 18845238]
71. Howard LA, Miksys S, Hoffmann E, et al. Brain CYP2E1 is induced by nicotine and ethanol in rat and is higher in smokers and alcoholics. *Br J Pharmacol*. 2003; 138:1376–86. [PubMed: 12711639]
72. Jin M, Kumar A, Kumar S. CYP2E1-mediated alcohol metabolism induces expression of CYP2A6 and CYP2E1 through oxidative stress-induced PKC signaling cascades in monocytes and astrocytes. *J Neuroimmune Pharmacol*. 2012; 7:S41.
73. Hamitouche S, Poupon J, Dreano Y, et al. Ethanol oxidation into acetaldehyde by 16 recombinant human cytochrome P450 isoforms: role of CYP2C isoforms in human liver microsomes. *Toxicol Lett*. 2006; 167:221–30. [PubMed: 17084997]
74. Hukkanen J, Jacob P III, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005; 57:79–115. [PubMed: 15734728]
75. Jin M, Kumar A, Kumar S. Ethanol-Mediated Regulation of Cytochrome P450 2A6 Expression in Monocytes: role of Oxidative Stress-Mediated PKC/MEK/Nrf2 Pathway. *PLoS ONE*. 2012; 7:e35505–12. The expression of CYP2E1 is regulated by alcohol metabolism-mediated oxidative stress through PKC/MEK/Nrf-2 pathway in monocytes/macrophages. [PubMed: 22530035]
76. Jin M, Earla R, Shah A, et al. A LC-MS/MS method for concurrent determination of nicotine metabolites and role of CYP2A6 in nicotine metabolism in U937 macrophages: implications in oxidative stress in HIV+ smokers. *J Neuroimmune Pharmacol*. 2012; 7:289–99. [PubMed: 21655912]
77. Walubo A. The role of cytochrome P450 in antiretroviral drug interactions. *Expert Opin Drug Metab Toxicol*. 2007; 3:583–98. Since all NNRTI and PI (HAART regimens) are metabolized by CYP3A4, and some of them also inhibit or induce CYP3A4, HAART medications pose serious challenges through drug–drug interactions. [PubMed: 17696808]
78. Pal D, Mitra AK. MDR- and CYP3A4-mediated drug-drug interactions. *J Neuroimmune Pharmacol*. 2006; 1:323–39. [PubMed: 18040809]
79. Pal D, Kwatra D, Minocha M, et al. Efflux transporters- and cytochrome P-450-mediated interactions between drugs of abuse and antiretrovirals. *Life Sci*. 2011; 88:959–71. CYP3A4 is the major enzyme in drug interactions between antiretrovirals and drugs of abuse, resulting in complex pharmacokinetic profiles of antiretroviral and undesirable response to HAART drugs. [PubMed: 20932495]
80. Lu C, Balani SK, Qian MG, et al. Quantitative prediction and clinical observation of a CYP3A inhibitor-based drug-drug interactions with MLN3897, a potent C-C chemokine receptor-1 antagonist. *J Pharmacol Exp Ther*. 2010; 332:562–8. [PubMed: 19889796]

81. Xu Y, Zhou Y, Hayashi M, et al. Simulation of clinical drug-drug interactions from hepatocyte CYP3A4 induction data and its potential utility in trial designs. *Drug Metab Dispos.* 2011; 39:1139–48. [PubMed: 21441468]
82. Feerman DE, Melinkov Z, Nanji AA. Induction of CYP3A by ethanol in multiple in vitro and in vivo models. *Alcohol Clin Exp Res.* 2003; 27:981–8. [PubMed: 12824820]
83. Ghosh C, Marchi N, Desai NK, et al. Cellular localization and functional significance of CYP3A4 in the human epileptic brain. *Epilepsia.* 2011; 52:562–71. [PubMed: 21294720]
84. Kumar S, Earla R, Jin M, et al. Effect of ethanol on spectral binding, inhibition, and activity of CYP3A4 with an antiretroviral drug nelfinavir. *Biochem Biophys Res Commun.* 2010; 402:163–7. Ethanol facilitates binding of nelfinavir with CYP3A4 and decreases the catalytic efficiency of CYP3A4 for nelfinavir metabolism. [PubMed: 20937259]
85. Kumar S, Kumar A. Differential effects of ethanol on spectral binding and inhibition of cytochrome P450 3A4 with eight protease inhibitors antiretroviral drugs. *Alcohol Clin Exp Res.* 2011; 35:2121–7. Ethanol differentially alters the binding and inhibition of CYP3A4 with protease inhibitors that have different physicochemical properties, which may differentially alter their metabolism. [PubMed: 21682753]
86. Kresina TF, Flexner CW, Sinclair J, et al. Alcohol use and HIV pharmacotherapy. *AIDS Res Hum Retroviruses.* 2002; 18:757–70. [PubMed: 12167267]
87. Flexner CW, Cargill VA, Sinclair J, et al. Alcohol use can result in enhanced drug metabolism in HIV pharmacotherapy. *AIDS Patient Care STDS.* 2001; 15:57–8. [PubMed: 11224929]
88. Kalichman SC, Amaral CM, White D, et al. Prevalence and clinical implications of interactive toxicity beliefs regarding mixing alcohol and antiretroviral therapies among people living with HIV/AIDS. *AIDS Patient Care STDS.* 2009; 23:449–54. [PubMed: 19413503]
89. Shibata N, Kageyama M, Kishida T, et al. Pharmacokinetic characterization of a human immunodeficiency virus protease inhibitor, saquinavir, during ethanol intake in rats. *Biopharm Drug Dispos.* 2003; 24:335–44. [PubMed: 14595702]
90. Shibata N, Kageyama M, Kimura K, et al. Evaluation of factors to decrease plasma concentration of an HIV protease inhibitor, saquinavir in ethanol-treated rats. *Biol Pharm Bull.* 2004; 27:203–9. [PubMed: 14758034]
91. Scopelliti F, Pollicita M, Ceccherini-Silberstein F, et al. Comparative antiviral activity of integrase inhibitors in human monocyte-derived macrophages and lymphocytes. *Antiviral Res.* 2011; 92:255–61. The activity of raltegravir and four other integrase inhibitors (MK-2048, L870, 810, IN2, and IN5) is relatively higher in macrophages than lymphocytes, perhaps because they are not metabolized by CYP3A4. [PubMed: 21867733]
92. Talakad JC, Kumar S, Halpert JR. Decreased susceptibility of the cytochrome P450 2B6 variant K262R to inhibition by several clinically important drugs. *Drug Metab Dispos.* 2008; 37:644–50. [PubMed: 19074527]
93. Tian G, Ghanekar SV, Aharony D, et al. Multiple inhibitor binding sites for transition state analogs and small molecule inhibitors. *J Biol Chem.* 2005; 278:28968–75. [PubMed: 12719412]
94. Hisaka A, Ohno Y, Yamamoto T, Suzuki H. Prediction of pharmacokinetic drug-drug interaction caused by changes in cytochrome P450 activity using in vivo information. *Pharmacol Ther.* 2010; 125:230–48. [PubMed: 19951720]
95. Mathias AA, West S, Hui J, Kearney BP. Dose-response of ritonavir on hepatic CYP3A activity and elvitegravir oral exposure. *Clin Pharmacol Ther.* 2009; 85:64–70. [PubMed: 18815591]
96. Croxtall JD, Perry CM. Lopinavir/Ritonavir: a review of its use in the management of HIV-1 infection. *Drugs.* 2010; 70:1885–915. [PubMed: 20836579]
97. Boffito M, Winston A, Jackson A, et al. Pharmacokinetics and antiretroviral response to darunavir/ritonavir and etravirine combination in patients with high-level viral resistance. *AIDS.* 2007; 21:1449–55. [PubMed: 17589191]
98. Brogan AJ, Mrus J, Hill A, et al. Comparative cost-efficacy analysis of darunavir/ritonavir and other ritonavir-boosted protease inhibitors for first-line treatment of HIV-1 infection in the United States. *HIV Clin Trials.* 2010; 11:133–44. [PubMed: 20736150]

99. von Weymarn LB, Chun JA, Hollenberg PF. Effects of benzyl and phenethyl isothiocyanate on P450s 2A6 and 2A13: potential for chemoprevention in smokers. *Carcinogenesis*. 2006; 27:782–90. [PubMed: 16364922]
100. Diedrich CR, Flynn JL. HIV-1/mycobacterium tuberculosis coinfection immunology: how does HIV-1 exacerbate tuberculosis? *Infect Immun*. 2011; 79:1407–17. [PubMed: 21245275]
101. Piggott DA, Karakousis PC. Timing of antiretroviral therapy for HIV in the setting of TB treatment. *Clin Dev Immunol*. 2011 Published online Dec 27, 2010. 10.1155/2011/103917
102. Patel N, Talwar A, Reichert VC, et al. Tobacco and HIV. *Clin Occup Environ Med*. 2006; 5:193–207. [PubMed: 16446262]

highlights

- Mild-to-moderate, as well as heavy alcohol consumption, is highly prevalent in HIV-infected individuals. This has been shown to increase HIV-1 replication and decrease the response to antiretroviral drugs, which leads to increased HIV-1 pathogenesis, disease progression and increased possibility of the development of neuroAIDS.
- CYP2E1 is known to play a critical role in alcohol metabolism and alcohol metabolism-mediated oxidative stress and toxicity in the liver and many other organs. This has been demonstrated to cause liver damage and pancreatitis, as well as oral and liver cancers in chronic alcohol users.
- CYP3A4 is known to play an important role in the metabolism of drugs, including PI and NNRTI to treat HIV-1 infection. Some PI and NNRTI are inducers of CYP3A4, while all PI are inhibitors of CYP3A4; these characteristics are responsible for drug–drug interactions between different antiretrovirals, as well as interactions between antiretrovirals and substances of abuse.
- CYP2E1 and CYP3A4 are the predominant CYPs expressed in monocytes/macrophages, astrocytes and to some extent in neurons. As cells of the monocyte/macrophage lineage are the major source of HIV-infected cells in the CNS, the presence of CYP enzymes in these cells is important in alcohol-mediated HIV-1 pathogenesis and neuroAIDS.
- CYP2E1 and CYP2A6 are regulated by the PKC/JNK/c-Jun and PKC/MEK/Nrf2 pathways, respectively, through alcohol metabolism-mediated oxidative stress in cells of the monocyte/macrophage lineage and astrocytes. The concurrent regulation of CYP2A6 and CYP2E1 by alcohol is intriguing and suggests an important role for CYP2A6 in nicotine-mediated oxidative stress in monocytes, macrophages and astrocytes in people who use both tobacco and alcohol.
- The current studies described, along with the studies proposed, would provide an opportunity to find novel therapeutic interventions, as well as for the determination of optimal drug dose adjustments for the treatment of HIV-infected alcohol users.

This box summarizes key points contained in the article.

Figure 1. Cells or organs that are involved in HIV-1 pathogenesis or neuroAIDS, as well as which are sites of action of antiretroviral therapy (ART) drugs

Some of these cells or organs, e.g., liver, are also involved in the metabolism of alcohol and ART drugs such as non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI).

Figure 2. Pathway showing the effect of alcohol on oxidative stress-mediated HIV-1 pathogenesis through CYP pathway

The PKC/MEK/Nrf-2 pathway is activated by oxidative stress generated from CYP2E1-mediated metabolism of alcohol. Nrf2 then activates the expression of CYP2A6 enzyme in these cells, which in turn metabolizes nicotine and produces oxidative stress.

MEK: Mitogen-activated kinase kinase; Nrf-2: Nuclear factor E2-related factor 2; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PKC: Protein kinase C.

Figure 3. Ethanol–CYP3A4–PI three-way interaction model with type II PI (TII-PI, blue), and spectrally unbound PI (UB-PI, red)

This model was derived from the data obtained using recombinant purified CYP3A4 enzyme. Ethanol (EtOH) differentially alters the inhibition of CYP3A4 by PI. Ethanol and type II PI interact simultaneously with CYP3A4 at the same site leading to a decrease in the IC_{50} of type II PI. Ethanol and spectrally unbound PI interact simultaneously with CYP3A4 at different sites leading to an increase in the IC_{50} of spectrally unbound PI. The altered CYP3A4–PI interactions by ethanol may lead to altered metabolism of PI and NNRTI.

Figure 4. Proposed strategy to study alcohol–ART interactions using recombinant CYP3A4 enzyme, HIV-infected macrophages and astrocytes, as well as hepatocytes and neurons

Alcohol–ART interaction may additively/synergistically enhance induction of CYP enzymes leading to increased metabolism of alcohol and ART thereby decreased bioavailability and efficacy of ART (red). This interaction is also expected to increase alcohol- and ART-mediated toxicity. Similarly, alcohol–ART physical interaction may differentially alter the binding and inhibition of CYP3A4 leading to altered metabolism of ART (increase or decrease), which may also alter the efficacy of ART and increase ART toxicity (blue). A decrease in the response to ART and an increased toxicity may eventually increase HIV-1 pathogenesis. In this case ART represents only PI and NNRTI.

ART: Antiretroviral therapy; MTB: Metabolites; ROS: Reactive oxygen species.