Published in final edited form as:

Mol Neurobiol. 2017 October ; 54(8): 5855–5867. doi:10.1007/s12035-016-0118-6.

Role of Autophagy in HIV Pathogenesis and Drug Abuse

Lu Cao¹, Alexey Glazyrin², Santosh Kumar³, and Anil Kumar^{1,*}

¹Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO 64108, USA

²Department of Pathology, School of Medicine, School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108

³Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA

Abstract

Autophagy is a highly regulated process in which excessive cytoplasmic materials are captured and degraded during deprivation conditions. The unique nature of autophagy that clears invasive microorganisms has made it an important cellular defense mechanism in a variety of clinical situations. In recent years, it has become increasingly clear that autophagy is extensively involved in the pathology of HIV-1. To ensure survival of the virus, HIV-1 viral proteins modulate and utilize autophagy pathway so that biosynthesis of the virus is maximized. At the same time, the abuse of illicit drugs such as methamphetamine, cocaine, morphine, and alcohol is thought to be a significant risk factor for the acquirement and progression of HIV-1. During drug-induced toxicity, autophagic activity has been proved to be altered in various cell types. Here we review the current literature on the interaction between autophagy, HIV-1, and drug abuse, and discuss the complex role of autophagy during HIV-1 pathogenesis in co-exposure to illicit drugs.

Keywords

HIV-1; Autophagy; Alcohol; Methamphetamine; Morphine

INTRODUCTION

Autophagy is a highly regulated vesicular trafficking pathway for the degradation of cytoplasmic components by lysosomes [1,2]. There are three types of autophagy, namely macroautophagy, microautophagy, and chaperone-mediated autophagy [3]. In this review, we will mainly focus on macroautophagy, the main autophagy pathway which has been extensively studied and characterized. Macroautophagy, which is usually referred to as “autophagy”, is mediated by a unique double-membrane organelle called autophagosome, where the excessive cellular components are engulfed and transported to lysosome. The

*Corresponding authors: A Kumar, Division of Pharmacology and Toxicology, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA. Tel: +1 816 235 1792; Fax: +1 816 235 1776; kumaran@umkc.edu; S Kumar, Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA, ksantosh@uthsc.edu.

outer membrane of autophagosome then fuses with a lysosome, and the inner vesicle is degraded by lysosomal enzymes. The substrates of autophagic degradation includes cellular organelles, protein aggregates, specific proteins, and even invasive pathogens [4]. Autophagy plays a critical role during deprivation condition, in which it recycles the captured components to sustain cellular energy level for survival [5]. Moreover, since the efficient clearance of excessive or damaged proteins and organelles is crucial for the normal performance of cells, dysregulation of autophagy may contribute to a variety of diseases, such as cancer, infectious diseases, and neurodegenerative disorders [6-8]. The process of autophagy was first reported in the 1960s, which showed an evidence of lysosomal digestion of intracellular components [9]. Ever since the first description, the research focus of autophagy has gradually transitioned from morphological identification and mechanistic dissection to studies on the role of autophagy during various physiological conditions in recent years.

Autophagy has been shown to play multiple roles in the immune system. Besides the inherent degradative function, autophagy pathway intersects with innate and adaptive immunity in antigen presentation, secretion pathways, and inflammation control [10-12]. Therefore, autophagy has long been recognized as a crucial self-defense mechanism. Disruption of autophagy by HIV-1 viral proteins has been discovered in various cell types which are infected or uninfected with HIV-1, such as CD4+ T cells and macrophages [13-15]. In recent years, mounting evidence has shown that autophagy is a critical process that is affected and utilized by HIV-1 during the viral life cycle [16]. The intersection of autophagy pathway and HIV-1 may facilitate the processing of viral proteins and assembly of virions. Moreover, since autophagy is responsible for the clearance of misfolded proteins during neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, defects in autophagic clearance is correlated with HIV-associated neurocognitive disorders as well. All of these evidence suggests the contribution of aberrant autophagy process to HIV-1 pathogenesis.

The manifestation of HIV-1 could be associated with drugs of abuse. Drug injection is a significant risk factor for acquisition of HIV-1. Moreover, the progression of HIV-associated neuronal pathogenesis is exacerbated among drug abusers, the mechanism by which it occurs is not fully understood. Emerging evidence revealed a cross-talk between drugs of abuse and HIV-1 viral proteins. Their shared mechanisms of injury, including production of proinflammatory cytokine and chemokine, oxidative stress, and apoptosis, accentuate the adverse impact of HIV. The role of autophagy is complex in HIV-1 pathogenesis in co-exposure to illicit drugs. There are shared pathways between autophagy and other stress-induced responses, including inflammation and immune responses [5].

In this review, we discuss the recent advances in the evolving understanding of the correlation between autophagy, drugs of abuse, and HIV-1. We describe the emerging evidence that the autophagy process is extensively targeted by the use of drugs, and in the context of HIV-1. The potential roles of autophagy in different cell types under administration of illicit drugs is described individually. In addition, we review the substantial findings regarding the involvement of autophagy in HIV-1 pathogenesis, in which, autophagy pathway is sequestered and utilized as a vehicle for viral replication. These

findings revealed novel understanding towards HIV-1 pathogenesis, and the potential of autophagic process for the development of a novel target for HIV-1 therapy.

AUTOPHAGY AND HIV-1

General Mechanisms of Autophagy

Autophagy is a highly regulated degradative pathway, which allows recycling of excessive cellular components under stress condition, thus protecting cells from death [17]. As a self-defense mechanism, autophagy can be induced by a variety of stimuli, ranging from nutrient deprivation to microbial invasion. As the major negative regulatory axis of autophagy, mammalian target of rapamycin (mTOR) is inhibited in response to stress signals, leading to activation of downstream UNC-51 Like Autophagy Activating Kinase-1 (ULK) complex, which contains ULK1, Atg13, and FIP200 [18]. The initial source of autophagosomal membrane could be nearby organelles such as endoplasmic reticulum (ER), mitochondria, and Golgi [19]. Under normal condition, the anti-apoptotic protein Bcl-2 binds to Beclin-1 via an inhibitory interaction [20]. Under starvation condition, however, Bcl-2 is dissociated from Beclin-1, and the activity of Beclin-1: hVps34: Atg14 leads to nucleation of autophagosome membrane. Further, the conjugation of Atg12 and LC3 causes expansion and enclosure of autophagosome [21]. The autophagosome containing captured components to be degraded finally fuses with a lysosome and forms an autophagolysosome, leading to degradation of the engulfed contents [17] (Figure 1).

Correlation between HIV-1 and autophagy (peripheral)

Autophagy plays important role in various cellular functions. In addition to maintaining intracellular nutrient level, autophagy is also found to be involved in infectious diseases, such as tuberculosis [22], where intracellular pathogens are targeted for autophagy degradation. There is increasing evidence that reveals the cross-talk between autophagy and HIV-1 (Table 1). The study by Espert et al has revealed that autophagy is triggered following binding of envelope glycoprotein (Env) to CXCR4 in T cells, and that autophagy is required for Env-induced apoptosis through CXCR4 [14]. It is the first observation that HIV-1 Env causes autophagy-dependent cell death via binding to cell surface receptor. Following this line, a study from the same group that focused on autophagy during HIV infection has revealed that autophagy is involved in HIV viral replication, and plays differential role in CD4 T cells and macrophages, further suggesting that autophagy is responsible for HIV-1 pathogenesis [13]. This study found that autophagy is repressed in infected CD4 T cells, while it is induced in infected monocytes/macrophages. In addition to Env, HIV-1 Tat can also be degraded by autophagy in CD4+ T lymphocytes, indicating the anti-HIV effect of autophagy [29]. Interestingly, HIV-1 has evolved so that it can counteract the degradation effect of autophagy. For example, HIV-1 Nef interacts with Beclin-1 and inhibits the maturation stage of autophagy, thereby avoiding the fate of being degraded [16]. HIV-1 Env has been shown to activate mTOR in dendritic cells, leading to exhaustion of autophagy and transferring of infection into CD4+ T cells [37]. These findings represent one of the pathological mechanisms that can be utilized as a potential therapeutic target for HIV-1.

Autophagy in HIV-associated Neurological Disorders

The dysregulation of autophagy in the central nervous system (CNS) has emerged as a feature of neuroAIDS. In post-mortem brains of patients with HIV-associated encephalitis, autophagy related proteins Beclin-1, Atg5, Atg7, and LC3II are found to be significantly increased, whereas no change in autophagy level is observed in HIV-1 patients without encephalitis [24]. This study has shown an increased amount of autophagosomes in CXCR4- or CCR5- tropic gp120 exposed neuronal cells as well. Another study performed on human neuronal cultures has shown that X4 HIV-1 suppresses autophagy leading to neuro-glial toxicity, whereas R5 HIV-1 only causes toxicity in neurons. Results of this study suggest that autophagy plays protective role against HIV-1 insult in human neuronal cultures [38]. Similarly, Tat has been found to cause a decrease in Beclin-1, LC3II, and p62 levels in neurons, suggesting that Tat may induce autophagic degradation by inducing fusion of autophagosomes and lysosomes [31]. Since autophagy is crucial for neuronal homeostasis, aberrant activation, and dysregulation of autophagy in HIV-1-induced neurotoxicity could be detrimental, leading to autophagic cell death.

Antiretroviral therapy and autophagy in HIV-1 systems

Ever since the introduction of highly active antiretroviral therapy (HAART) in the 1990s, the incidence of HIV-associated dementia (HAD), the most severe form of HIV-associated neurocognitive disorders (HAND), has been significantly decreased. However, the less severe form, namely minor neurocognitive motor disorder (MCMD), remains to be a significant problem [39,40]. Despite antiretroviral effects, the use of antiretroviral therapy (ART) drugs has been associated with the development of several adverse events, such as increased cardiovascular risk [41], hepatotoxicity [42], and renal toxicity [43]. Emerging evidence showing activated autophagy, along with the use of ART, suggests that autophagy could be another mechanism that is affected by these classes of drugs.

Among ART drugs, the non-nucleoside reverse transcriptase inhibitor efavirenz has been associated with dysregulation of autophagy and ER stress in multiple studies. In these cases, efavirenz has been reported to regulate autophagy that differentially depends on the dose and cell type. For example, a study performed on human brain endothelial cells has associated efavirenz with decreased level of autophagy, which contributes to the toxicity of efavirenz [44]. While another study on human hepatic cells has shown that moderate levels of efavirenz activates autophagy, whereas higher concentrations can cause blockade of autophagic flux, leading to cellular damage [45]. Other than efavirenz, protease inhibitors atazanavir and saquinavir have been found to cause induction of autophagy, which contributes to toxicity of these drugs [46,47]. In conclusion, as an important mechanism for maintaining cellular homeostasis, the level of autophagy is altered upon treatment of antiretroviral drugs. As dysregulation of autophagy often correlates with increased ER stress and even apoptosis, this effect can be implicated in pharmacological damage associated with ART.

As discussed in above sections, emerging studies reveal the role of autophagy in HIV-1 infection, which shows the importance of autophagy in HIV-1 pathogenesis, especially to the patients who are on ART. Disruption of autophagy is never an isolated event as it is most

likely accompanied by a variety of pathological consequences, such as cytokine production, ER stress, and apoptosis. Many questions, however, remain unexplained regarding the correlation between HIV-1 and autophagy, such as differential regulation and role of autophagy in various cell types. Furthermore, due to the fact that the use of substances of abuse are highly prevalent among HIV-1 patients, the role of autophagy in HIV-1 progression becomes more complicated in these patients.

AUTOPHAGY AND DRUGS OF ABUSE

Drugs of abuse in general

Drugs of abuse (DOA), such as methamphetamine (METH), cocaine, morphine, and alcohol, are associated with toxic impact on almost every system in humans. Besides their inherent risks, drugs of abuse have been reported to compromise the immune system, which makes the abusers more susceptible to infection, leading to a broad range of health-related issues, such as HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) infection [48]. In addition to that, drug abuse and mental illness co-exist in most cases, which can trigger risky behaviors, such as suicide, violence, and unprotected sex. The symptomatic effects of drugs of abuse results from alteration in levels of neurotransmitters and function of their receptors. Opioids, namely morphine, bind to specific opioid receptors, especially μ , κ , and δ receptors, in the central and peripheral nervous systems. Psychostimulants, such as METH and cocaine, cause accumulation of neurotransmitters norepinephrine, dopamine, and serotonin in the brain. In addition to the fact that alcohol can be metabolized in the liver and extrahepatic cells, it can enter the brain and alter the function of GABA, NMDA, and acetylcholine receptors. In conclusion, drugs of abuse induce neurotoxicity via complex mechanisms. In recent years, the question of involvement of autophagy has been raised in context of drugs of abuse, which is evident from the presence of multi-membrane vacuoles with autophagic characteristic that have been observed in various cell types.

Role of autophagy in DOA-induced toxicity

1. Methamphetamine—The involvement of autophagy in neurons by exposure of METH was initially characterized in 2002 [49]. In a study performed to examine METH-induced neurodegeneration, autophagic vacuoles were observed in a time-dependent manner in mouse primary midbrain neuronal cultures [49]. Following this line, the role of autophagy under low doses of METH treatment has been studied [50]. Presence of autophagosomes were observed following 1 μ M of METH treatment in PC12 cells, along with an increase in the levels of Beclin-1 and LC3II. Inhibition of autophagy precipitated caspase-dependent cell death in METH-treated cells, suggesting that METH plays a protective role against apoptosis following short-term METH treatment. An *in vivo* study performed on METH-treated rats revealed that METH caused elevated LC3II and ubiquitinated protein levels, and PKC δ cleavage [51]. Inhibiting autophagy rendered rat dopaminergic cells prone to METH-induced apoptosis through caspase-3 activation. Results from this study confirmed the protective role of autophagy against METH-induced apoptosis. In another study performed on endothelial cells, κ -opioid receptor is found to be responsible for METH-induced autophagy [52]. All of the above studies have demonstrated the pro-survival role of autophagy against METH-induced cytotoxicity.

Despite the protective role of autophagy that has been reported by multiple groups, several studies have shown that autophagy is involved in METH-induced neurotoxicity in some cell types. Inhibition of autophagy by activating mTOR has been shown to reduce METH-induced cell death in human neuroblastoma SK-N-SH cells [53]. This study suggests that autophagy is involved in METH-induced neurotoxicity, and its effect can be reversed by administration of melatonin. Another antioxidant, taurine, has been shown to cause attenuated autophagy activity level in PC12 cells in co-exposure to METH, leading to increase in cell viability [54]. The findings that these antioxidants attenuate autophagy level and METH-induced cell death reveals the delicate balance between autophagy and apoptosis under stress condition, which according to previous studies, is regulated by Beclin-1: Bcl-2 complex [55]. The fact that inhibiting autophagy abolished METH-induced cell death does not necessarily conflict with the role of autophagy as an initial defense mechanism in the early stage in response to METH-induced stress.

2. Morphine—Autophagy has been implicated as one of the possible mechanisms that contribute to morphine-mediated cell death. Preceding morphine itself, morphinone, one of the active metabolites of morphine, has been found to induce non-apoptotic cell death in human promyelocytic leukemia cells [56]. This study showed that morphinone exhibits slightly higher toxicity towards human tumorous cell lines compared with normal human cell lines, and the cell death is independent of caspase-8 or 9. Besides that, formation of autophagosome was found, and blocking of autophagy pathway attenuated morphinone-induced cytotoxicity. Results of this study suggest that autophagy may contribute to the unidentified cell death mechanism mediated by morphinone. Although the presence of autophagy has been characterized, this study did not go further into the role of autophagy in morphinone-induced cytotoxicity. To further explore morphine-induced neurotoxicity, a study performed on human neuroblastoma SHY-5Y cells has shown that morphine induces autophagy in a pathway that is dependent on opioid receptor, Beclin-1, and Atg5 [57]. Despite the assumption that autophagy may function as a pro-death mechanism, this study showed that inhibiting autophagy exacerbates morphine-induced cell death. A recent *in vivo* study has shown that activation of autophagy led to a decrease in neuronal cell death, and it suppressed inflammation in the hippocampus, and thus mitigated the memory impairment induced by morphine [58].

Parallel to discovering the role of autophagy to morphine-induced neurotoxicity, some recent studies have focused on physiological processes that are closely regulated by autophagy. In one such process, chronic morphine use has been associated with mitochondrial dysfunction [59]. Following this line, the reduction in the mitochondrial DNA copy number during morphine addiction has been shown to be mediated by autophagy [60]. One of the primary functions of autophagy is to serve as a host-defense mechanism against infection. In effort to illuminate the role of autophagy in innate immunity, a recent study has found that morphine potentiates lipopolysaccharide-mediated autophagy activation [61]. Nevertheless, the maturation of autophagosome is inhibited, leading to decreased bacterial clearance. The findings of this study is closely correlated with the increased susceptibility of drug abusers to infection.

3. Cocaine—Compared to other substances of abuse, there is limited report on cocaine-induced autophagy. Researchers have been seeking to connect uncontrolled autophagy with cocaine-induced neurotoxicity. When treated with cocaine, extensive autophagosome formation and increased level of autophagy marker have been found in astrocytes, primary rat microglial cells, and primary neuronal cerebrocortical cultures [62-64]. In these studies, an increased level of autophagy has been associated with activation of ER stress pathway and release of inflammatory factors, such as TNF, IL-1 β , IL-6, and CCL2. Chronic treatment of cocaine leads to cell death, which can be attenuated by blocking the autophagy pathway using specific inhibitors, suggesting the involvement of autophagy in cocaine-induced cytotoxicity. Since there is no complete reverse in cell death, these results suggest that besides autophagy, several other cell death mechanisms are also responsible for the cell death caused by chronic cocaine-exposure [62].

4. Alcohol—The liver is the organ that is primarily impacted by alcoholism, since majority of alcohol is metabolized in the liver [65]. Binge drinking of alcohol brings multiple physiological concerns, such as insulin resistance, mitochondrial damage, and apoptotic and necrotic cell death [66,67]. Since autophagy is a critical regulator of intracellular metabolism, perturbation in the cellular homeostasis by alcohol may lead to dysregulation of autophagy. Several initial studies have shown that autophagy is suppressed in alcoholic liver disease. For example, less density of autophagosome and autolysosome were found in the liver cells of rats fed with alcohol compared with control rats [68]. The lowering of the protein degradation rate in alcohol-fed rats caused increased accumulation of long-lived proteins [69]. In addition to liver cells, autophagy process is also suppressed in human monocytic U937, CD4 Jurkat, MCF-7 cells, cardiomyocytes, and cortical neuroepithelial progenitors, following alcohol exposure [70-72]. This evidence suggests that administration of alcohol leads to loss of autophagic degradation function, rendering the cells susceptible to apoptotic cell death. Regarding the mechanism of suppression in autophagy, it has been proposed that alcohol inhibits AMP-activated protein kinase (AMPK), an upstream regulator of autophagy, leading to suppression of the downstream autophagy signaling pathway via mTOR [73]. Another proposed explanation is that the vesicular movement in hepatocyte is disrupted by alcohol, thereby blocking the formation of autophagosome [74]. The pathological outcome of decline in autophagy activity includes accumulation of unnecessary proteins and failure in clearance of cellular organelles damaged by alcohol, such as mitochondria [75-77].

Nevertheless, several recent studies have suggested induction of autophagy following alcohol consumption. Binge alcohol treatment induces autophagy in the liver of mice and primary hepatocytes [78]. In this study, autophagy plays a protective role against alcohol-induced hepatocyte apoptosis and liver injury. Alcohol-induced autophagy is found to be selective for removal of damaged mitochondria and lipid droplets, thus mitigating steatosis and apoptosis [79,80]. Furthermore, induction of autophagy is also responsible for the alleviation of ROS production thus ameliorate alcohol-induced neurotoxicity [81].

The conflicting role of autophagy under alcohol exposure revealed in recent years depends strongly on the dose of alcohol administration and cell type. However, the consensus among these studies is that autophagy plays a critical role in alcohol-induced cytotoxicity,

especially in the clearance of damaged organelles and toxic side products during alcohol metabolism.

AUTOPHAGY IN HIV-1 WITH CO-EXPOSURE OF DOA

Drug abuse among HIV-infected individuals

Although HIV prevalence in injection drug users (IDU) has declined in recent years, drug abuse remains one of the major factors that contributes to HIV-1 transmission and progression. Illegal drug use is considered the second most common route for HIV-1 transmission via shared needles and unprotected sexual behaviors [82]. The global estimate of 15 to 64 year old HIV patients who inject drugs is 18.9% [83]. In the United States, there are striking racial and ethnic disparities among drug injectors, with African Americans counting for 50% of the new HIV infections with injected drug use in 2010 [84]. Drug abusers, especially IDU, are far more likely to be co-infected with the HCV, with 50%-80% carrying both HIV and HCV [85,86]. Besides injection drug users, the prevalence of HIV among non-injection drug users is of growing awareness because of high-risk behaviors and overlapping social and sexual network with HIV-infected injection drug users [87,88].

Role of DOA in HIV-1 pathogenesis

In addition to increased risk of contracting HIV due to impaired judgment towards risky behaviors, DOA can also negatively affect treatment and maintenance of HIV in many ways (Table 2). Drug abuse may have direct or indirect biological effects that facilitate the progression of HIV-1 infection. Firstly, certain DOA are associated with immunosuppressive effect, rendering drug users more prone to virus infection. Studies have found that morphine suppressed T cell activation in the lymph node, paralleled with a decrease in activation levels of T cells in peripheral blood in nonhuman primates [126]. Methamphetamine has been shown to accelerate the premature demise of activated T cells [127], and reduce the number of dendritic cells and natural killer cells [128,129]. Other than that, excessive reactive oxygen species is produced when astrocytic cells are treated with methamphetamine via CYP2E1, leading to apoptotic cell death [95]. *In vitro* studies have shown that cocaine causes increased HIV infection in human PBMC [130,131]. Also, drug use has been associated with differential disease progression. Poundstone et al. reported that the increase in the disease-free survival time was significantly less among injecting drug users compared with non-drug users in HAART era [132]. For injecting drug users, there seems to be a greater barrier to the application of HAART [133]. The underlying factors that cause such a difference in the therapeutic outcomes is still under discussion. Nevertheless, there is evidence showing that injecting drug users exhibit poor adherence to HAART. It has been reported that active drug use, especially cocaine, was significantly associated with less overall adherence to HAART along with poor viral suppression comparing with non-users [134].

Role of DOA in HAND

As HAND remain to be a significant health problem in HAART era, the concomitant use of drugs has long been recognized as a major risk factor that contributes to neurocognitive dysfunction in HIV-positive individuals. The pathophysiology of HAND is complex, and is

further exacerbated and complicated by use of drugs. Compared with non-users, HIV patients who are drug users exhibit increased frequency of HIV-encephalitis [135]. Some of the drugs, such as cocaine and methamphetamine, exert addictive and reinforcing effects via increased extracellular dopamine level in the brain, while altered activity of dopamine and dopamine receptors has been implicated in the pathogenesis of HIV-1 in terms of viral entry and replication [112,136]. Other factors that facilitate HIV viral entry includes disruption in the integrity of blood-brain barrier (BBB). It has been reported that morphine administration, along or in combination with HIV-1 Tat, caused decreased expression of tight junction proteins ZO-1 and occludin, leading to enhanced transendothelial migration across the BBB [103]. Other than that, there is a profound evidence correlating DOA with increased levels of cytokine/chemokine, oxidative stress, and activation of metalloproteinase, in the context of HIV-1 [137-140]. All of these findings suggest that DOA are strongly associated with enhanced risk of HIV-induced brain injury.

Involvement of autophagy in DOA mediated HIV pathogenesis and HAND

The neurotoxicity associated with HIV is accelerated and exacerbated by drug abuse. Although disruption of autophagy has been documented in neuronal cells during neurodegenerative diseases, the involvement of autophagy in HIV-associated neurotoxicity under drug exposure is relatively unknown [141-143]. Autophagy is considered to be suppressed during HIV-1, while several studies have suggested the differing roles of autophagy in the pathogenesis of HIV-1 with co-exposure to DOA. One such study has examined the autophagy pathway using HIV-infected human primary microglial cells [105]. Exposure of morphine was found to decrease virus-induced Beclin-1 expression and autophagosome formation by increasing intracellular pH, and that the interaction between morphine and HIV-1 is a Beclin-1-independent process. Another study on morphine was performed by the same research group, using post-mortem brain tissues from HIV-1 positive patients with neurocognitive impairment (NCI) $\pm$ HIV-encephalitis (HIVE) [144]. This study showed more complex results, in which, autophagy genes and related proteins are differentially regulated during different stages of HIV-1. Another drug, methamphetamine, has been shown to cause increased amount of autophagosome in combination with HIV-Tat [97]. Activation of autophagy showed a protection effect against a long-term drop in the ATP level during cART in combination with methamphetamine or HIV-1 gp120 [145]. In a recent study we have examine the cumulative effect of methamphetamine and HIV-1 gp120 on autophagy induction. Our results indicate that concurrent exposure with multiple doses of methamphetamine and gp120 cause an increase in LC3II level and the amount of autophagosome. We have also concluded from this study that the autophagy was mediated by signaling pathway involving mTOR, Beclin-1, Atg5, and Atg7. We further showed that autophagy inhibitors exacerbated gp120/methamphetamine-mediated cell death suggesting a protective role of autophagy in astrocyte death [146]. In spite of the fact that only limited knowledge is available regarding cumulative effect of DOA and HIV-1, it is logical to believe that autophagy plays an important role in HIV-1 pathogenesis.

CONCLUSION AND FUTURE PROSPECTIVE

In summary, the research reviewed in this article makes a strong case for autophagy to be a critical target for manifestation of DOA and HIV-1. The role that autophagy plays in the consequences of HIV-1 infection, in the context of illicit drugs, is an area that is largely unexplored. The complex effects of autophagy contributes to HIV viral replication and immune evasion. Autophagy pathway is extensively affected in the toxicity mediated by DOA, while its role varies with administration of different drugs and cell types. The principle function of autophagy is to maintain intracellular homeostasis and normal activity of cells, and it has been proved that autophagy savage cells from drug-induced toxicity, and that blockade of autophagy degradation renders cells susceptible to apoptotic cell death. Nevertheless, excessive autophagy induced by drug administration leads to cell death, which can be reversed by blocking autophagy pathway. Recent studies showed conflicting evidence on whether autophagy pathway is activated or inhibited during alcohol treatment. One possible explanation is that different administration modes of alcohol, such as short-term or chronic consumption, may exert different effect on normal cellular function.

New insights into the pathogenesis of HIV-1 are being revealed by the findings that autophagy pathway is targeted and utilized during viral life cycle. Though traditionally viewed as a degradative pathway, autophagy process can also play an anabolic role and promote certain steps in HIV biosynthesis. Still, important questions remain in the current knowledge of autophagy in the whole scenario of drug use in the HIV system. Since both illicit drugs and HIV-1 proteins evoke a series of molecular events, whether they intersect with each other, and their functional outcome is still unknown. As autophagy is a stress response that cannot be quantified, the switch that determines whether autophagy is protective or deleterious is also unknown. Finally, since various cellular responses are mediated by HIV-1 and illicit drugs, such as increased inflammation, oxidative stress, ER stress, and enhanced viral replication, the balance and interaction between autophagy and these pathological events may eventually determine the fate of the cells.

In summary, the importance of autophagy in pathogenesis of HIV in co-exposure of illicit drugs is becoming increasingly clear. Our understanding of the correlation between autophagy and other cellular mechanism is still quite primitive. The interface between these processes will be a fruitful scientific area, and study of autophagy in various cell types may provide novel insight into pathogenesis of HIV-1 and potential therapeutic strategies for the treatment of HIV.

Acknowledgments

Grant support:

This work was supported by grants from National Institute on Alcohol Abuse and Alcoholism AA020806 (AK) and AA022063 (SK).

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Figure 1. Schematic overview of the signaling pathways leading to drug-induced autophagy Autophagy is a catabolic process in which excessive or damaged proteins and cellular organelles are degraded in autophagosomes. Drugs bind to surface receptors such as opioid, mGluR5, and sigma-1 receptors, leading to series of downstream molecular event. The mammalian target of rapamycin (mTOR) suppresses autophagy pathway when nutrient condition is adequate for normal cellular function. In the context of drugs, ULK1 complex is activated upon suppression of mTOR, leading to initiation of autophagy. The formation of autophagosome requires PI3KIII/Beclin-1 complex. The conjugation of Atg16, Atg12, and LC3 is critical for the elongation of the vesicular membrane. LC3II, the lipidated form of LC3, presents on both the outer and inner membrane of autophagosome, therefore is widely used as an autophagic marker protein. The mature autophagosome then fuses with a

lysosome to form an autophagolysosome. The contents that is sequestered by autophagosome are degraded by lysosomal enzymes.

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Table 1

Correlation between HIV-1 proteins and autophagy in different cell types.

HIV-1 protein	Cell type	Correlation with autophagy	References
Env	Neurons and uninfected T cells	Env binds to CXCR4 and induces autophagy triggers cell death.	[14,23,24]
	Infected T cells	Autophagy is inhibited to promote viral replication.	[13,25]
	Cardiomyocytes	gp120 induces autophagy via NMDA receptor.	[26]
Tat	Uninfected macrophages/monocytes	Tat blocks autophagy through Src-Akt and STAT3 signaling.	[27]
	Human macrophages	Tat suppressed IFN- γ -induced autophagy.	[28]
	T cells	Tat is selectively degraded by autophagy.	[29]
	Glial cells	Tat induces autophagy through enhancement of BAG3 protein level.	[30]
	Neurons	Tat induces autophagic degradation by promoting fusion process.	[31]
Gag	Macrophages	Gag-derived proteins colocalized and interacted with the autophagy factor LC3, and autophagy promoted productive Gag processing.	[16]
Nef	Macrophages	Nef inhibits autophagy maturation through interactions with Beclin-1	[16]
	HeLa cells/hepatocarcinoma	Nef induces autophagy through an IRGM dependent pathway.	[32]
	Bone marrow mesenchymal stem cells	Nef treatment causes early inhibition of autophagy, thus inducing MSC senescence.	[33]
	Infected macrophages	Nef modulates TFEB localization and alters autophagy degradation.	[34]
	Human astrocytes	Nef mimics Baf A1 and blocks the formation of autophagolysosome.	[35][36]

Table 2

Interactions between drug abuse and HIV-1.

Drugs	Risk for HIV infection	ART adherence	ART efficacy	HIV pathogenesis	HAND	Apoptosis	Autophagy
METH	↑ [89]	↓ [90]	↓ [91]	Increased viral load [91] Accelerated CD4+ T cell decline [92] Immunomodulation [93]	Modulation of neurotransmitters [94] Increased oxidative stress [95] Neuroinflammation [96]	↑ [95]	↑ [97]
Morphine	↑ [98]	— *	— *	Immunomodulation [99] Increased viral replication [100]	Neuroinflammation [101] Increased oxidative stress [102] Increased BBB permeability [103]	↑ [104]	Limited interaction [105]
Cocaine	↑ [106]	↓ [107]	↓ [108]	Enhanced viral replication [109] Decline in CD4+ T cells [110]	Impaired neurogenesis [111] Modulation of neurotransmitters [112] Alteration in BBB integrity [113]	↑ [114]	↑ [115]
Alcohol	↑ [116]	↓ [117]	↓ [118]	Enhanced viral replication [119] Acute exposure: Suppression of immune response [120] Chronic exposure: Increased cytokine production [121]	Neuroinflammation [122] Modulation of neurotransmitters [123] Astroglial activation [124]	↑ [125]	* —

* No information available.