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# HIV-1, Drug Addiction, and Autophagy

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## Abstract

Despite the dramatic success of combined antiretroviral therapies (cART) in controlling peripheral virus replication, the prevalence of HIV-1-associated neurocognitive disorders (HAND) is on a rise as infected individuals continue to live longer. Almost half of the infected individuals on ART develop HAND, out of which at least 30% suffer from a comorbid condition of substance abuse. Involvement of autophagy has been implicated not only in HIV-1 infection of the CNS but also in CNS cells exposed to drugs such as amphetamine, opiates, and cocaine, contributing in turn, to cellular dysfunction. HIV-1 is known to interfere with the autophagy pathway, resulting in turn to upregulation of HIV-1 replication. Specifically, different HIV-1 proteins such as TAT, gp120, and Nef have been shown to act on various stages of autophagy such as initiation and maturation and to affect overall autophagy levels. Whether or not abused drugs and HIV-1 can cooperate to dysregulate autophagy, however, remains unclear. This chapter is focused on identifying the molecular mechanism(s) underlying HIV-1 (proteins) and cocaine, opiate, methamphetamine-mediated impairment of autophagy. Such effects could underlie the synergistic effects of HIV-1 and abused drugs in exacerbating symptoms of HAND.

**Keywords:** HIV-1, TAT, gp120, drug addiction, cART, autophagy

## 1. Introduction

Since the advent of combined antiretroviral therapy (cART), human immunodeficiency virus (HIV-1) infection has transformed into more manageable and controllable chronic disease analogues to diabetes [1]. HIV-1-infected individuals on cART are living longer compared to HIV-1-seronegative controls. Despite the dramatic success of cART in controlling viremia and increased longevity, various comorbidities involving multiple organs including the brain are on a rise in the infected individuals [2, 3]. In the context of CNS, there is an increased prevalence

of HIV-1-associated neurocognitive disorders (HAND) due to the extended life expectancy. HAND is composed of various entities of neurocognitive impairments ranging from asymptomatic to milder forms of motor disorders. Increased activation of microglia and astrocytes (neuroinflammation) and synaptodendritic injury are the emerging hallmark features of HAND. Although multiple factors and pathways have been proposed in the development of HAND, the exact molecular mechanisms underlying the pathogenesis of HAND in the era of cART remain elusive.

Autophagy is a highly conserved cellular process that is present in all eukaryotic cells. Autophagy allows the orderly degradation and recycling of cellular components [4]. During this process, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membraned vesicle known as the autophagosome. The autophagosome fuses with a lysosome and the contents are degraded and recycled. Autophagy can be broadly divided into several stages: initiation, elongation, autophagosome formation, and maturation. Each stage is tightly and sequentially regulated by multiple autophagy-related proteins, to ensure the completion of whole process (autophagy flux).

It is well recognized that drug abuse serves as a significant risk factor for acquiring HIV-1 infection and has been implicated in worsening the symptoms of HAND [5, 6]. HIV-1-positive individuals with a history of abused drug exposure exhibit severe cognitive and behavioral dysfunction compared to those without exposure to abused drugs. Clinical evidence points to increased neuroinflammation, severe neuronal injury, and increased viral loads in the brains of HIV-1-positive patients with a history of drug addiction compared to the brains of infected individuals that were drug naive [5, 6]. Abused drugs including cocaine, methamphetamine, and opiates have been shown to interact with the autophagy pathway in various kinds of cells including microglia, astrocytes, and neurons to alter their homeostasis. Dysfunction of these three cell types plays critical roles in the pathogenesis of HAND.

In this chapter, we focus on the intermingled relationship between HIV-1, abused drugs, and autophagy. HIV-1/HIV-1 proteins and drugs of abuse, that have been shown to interact with various stages of autophagy pathway, can synergistically lead to autophagy dysregulation, which, in turn, contributes to accelerated pathogenesis of HAND. Understanding and clarification of the complex interplay of HIV-1, abused drugs and HAND could set a stage for future development of novel target(s) as alternative therapeutic approaches to ameliorate HAND in HIV-1-infected individuals on cART.

## 2. HIV-1, HAND, and cART

In 1983, HIV-1 was first isolated and identified as a lentivirus (a subgroup of retrovirus) that was the causative agent for acquired immune deficiency syndrome (AIDS). In the pre-antiretroviral therapy era, the average survival time following infection with HIV-1 was estimated to be 9–11 years, depending on the HIV-1 subtype [7]. In 1996, the introduction of combined antiretroviral therapy (cART) changed the course of the HIV-1 epidemic. In the current era of cART, HIV-1 infection has become a more controllable and manageable disease,

with increased patient longevity [1, 8]. Clinical studies have shown that in patients with well-controlled viremia, the CD4 numbers are relatively maintained in a normal range (400–1600/mm<sup>3</sup>) and also that the function of immune system is well preserved with extended periods of undetectable viral loads.

HAND is composed of neurological complications that range from asymptomatic neurocognitive impairment (ANI) to milder forms of cognitive impairment (MNI). While severe complications of CNS such as HIV-associated dementia (HAD) have declined in the post-cART era, emergence of milder cognitive motor disorders is actually on rise. In fact, as HIV-1-infected individuals continue to live longer lives, it is estimated that up to 50% of those individuals still continue to display varying degrees of neurocognitive impairment, which could impact their quality of life, leading to increased healthcare burden.

Various theories are extant about the mediators that regulate pathogenesis of HAND. For example, it has been implicated that direct injury caused by HIV-1 and its associated proteins such as the envelope gp120, transactivator of transcription (TAT), and Vpr can mediate damage in the CNS. Furthermore, indirect damage caused by proinflammatory cytokines/chemokines and chronic, sustained immune activation in the CNS can all play key roles in the pathogenesis and progression of HAND [9, 10]. It is now well accepted that events secondary to HIV-1 infection (inflammation) are critical for CNS damage associated with HAND [10]. Other factors have also been shown to contribute to HAND development. For example, factors such as inefficient CNS penetration of cART and/or emergence of latent virus reservoirs can also be attributable factors to the pathogenesis of HAND [11, 12]. Additionally, it is now becoming well recognized that HAND is prevalent in infected individuals abusing recreational drugs. In fact, substance abuse and co-infection with hepatitis C are recognized comorbidities of HIV infection. In summary, while treatment with cART has reduced the incidence of the severe form of CNS impairment such as HAD, low-level ongoing inflammation in the periphery and in the CNS and HAND is emerging as newer comorbidity of HIV infection.

### 3. HIV-1 and autophagy

Autophagy and the innate immune responses are highly conserved evolutionarily processes in virtually all the eukaryotic cells [4]. Autophagy is an important player in various diseases such as cancer and neurodegenerative disorders [4]. At the cellular level, HIV-1 can infect dendritic cells (DC), monocytes, and CD4<sup>+</sup> T cells in the periphery and microglia and astrocytes (limited infection) in the CNS. Depending on the cell type, HIV-1 can either usurp certain autophagy-related proteins or block autophagy flux to invade the host immune response, thereby exacerbating HIV-1 replication and transmission [13].

In DCs, HIV-1 impairs autophagy to subvert host immunity and enhance trans-infection. The efficiency of DCs being infected by HIV-1 heavily depends on their origin and activation state [14]. Previous reports have demonstrated that immature, resting DCs are more readily infected with HIV-1 than mature, activated DCs that are induced either by TNF- $\alpha$  or by poly I:C [14, 15]. Of note, while immature DCs seem to be more easily infected with HIV-1, mature DCs are

more efficient at mediating trans-infection to CD4<sup>+</sup> T cells without themselves getting infected [14]. The idea that autophagy is a mechanism that protects against HIV-1 infection originated from the fact that treatment of DCs with either TNF- $\alpha$  or poly (I:C) led to the activation and upregulation of autophagy suggests thereby that elevated levels of autophagy degrade the incoming virions in mature DCs [15]. Another study indicated that viral Env downregulated autophagy through mTOR activation, thereby protecting the virus from autophagy-mediated degradation, ultimately leading to increased trans-infection to CD4<sup>+</sup> T cells [16]. These results reveal a mechanism by which HIV-1 limits autophagy in DCs to target immune function leading, in turn, to increased transmission.

HIV-1 is known to infect macrophages, without a negligible change in cell numbers [17]. The role of autophagy in viral replication in the macrophages, however, remains poorly explored. In one study, it was shown that autophagic vacuoles were increased in infected macrophages. The mechanism underlying this phenomenon involved inhibition of the maturation stage of autophagy by HIV-1 factor Nef, via its association with the autophagy regulator Beclin-1 [18]. Thus, HIV-1 can exploit early stages of autophagic signaling in the macrophages for biogenesis and egress, while concomitantly also inhibiting the maturation stages of autophagy to prevent its own degradation. One of the key hallmark features of HIV-1 infection is a significant depletion of CD4<sup>+</sup> T cell in patients that are treatment naive [19, 20]. Previous investigations have addressed the effects of HIV-1 protein Env on bystander T-cell death [20]. Although autophagic vacuoles (AV) were increased in these bystander T cells, the exact role of autophagy contributing to cell death still remains elusive. Since the autophagic process involves the capture and degradation of activated inflammasome complexes, increased numbers of AV in the bystander CD4<sup>+</sup> T cells could thus imply an attempt by the cell to upregulate autophagy as part of an anti-inflammatory response. The failure of autophagy to protect these bystander cells from inflammasome activation could eventually lead to cell death. Thus, while autophagy has no direct role in CD4<sup>+</sup> T-cell death, it can be envisioned as a secondary, compensatory mechanism that is an attempt at salvaging the cells from apoptosis [21].

Microglia is the primary immune-competent cells of the brain. Similar to peripheral immune cells, HIV-1 and viral proteins also modulate autophagy levels in microglia. A recent study has demonstrated that expression levels of autophagy markers such as Beclin-1, ATG5, LC3-II, and p62 were significantly altered in microglia from HIV-1-infected individuals with NCI  $\pm$  HIV-1 encephalitis (HIVE) [22]. Autophagy dysregulation could thus be associated with the microglial activation status. Another investigation provided a more direct evidence focusing on the role of HIV-1 infection on microglial autophagy [23]. In this study, it was shown that in primary human microglia infected with macrophage-tropic HIV-1SF162 strain, there was increased protein expression of Beclin-1 and LC3-II. In these infected cells, accumulation of LC3 reporter RFP<sup>+</sup> GFP<sup>+</sup> (yellow) puncta suggested that HIV-1 infection triggered autophagosome formation without promoting protein degradation by the lysosomes. These findings imply that HIV-1 can usurp autophagy pathway to promote its own replication. Increasing autophagy flux could thus serve as a potential therapeutic approach against HIV-1 infection in microglia. Similar to microglia, dysregulation of autophagy in HIV-1-infected astrocytes has also been reported [24].

Unlike the microglia and astrocytes, HIV-1 does not infect the neurons, primarily due to lack of CD4<sup>+</sup> receptor in these latter cells. Neurons, however, are susceptible to the toxic effects of HIV-1 proteins TAT and gp120, which have been shown to dysregulate autophagy, leading, in turn, to neurotoxicity and/or neuronal injury including synaptodendritic injury. A seminal report [25] investigated the role of autophagy in microglia-induced neurotoxicity in primary rodent neurons. These authors demonstrated that conditioned media from simian immunodeficiency virus (SIV)-infected microglia inhibited autophagy in rodent neurons, leading to decreased neuronal survival. It is likely that a combination of HIV-1 proteins (TAT/gp120) and/or multiple inflammatory mediators released from the infected cells could be contributing to the dysregulation of autophagy. Another study has provided evidence that HIV-1 protein TAT can impair the endolysosome structure and function and ensuing autophagy in the neurons [26]. In this study, it was shown that following the treatment of primary cultured rat hippocampal neurons with HIV-1 TAT, neuronal viability was significantly decreased. The authors reported significant changes in the structure and membrane integrity of endolysosomes, endolysosome pH, and autophagy flux, which were responsible for neuronal death. In agreement with these findings, report by Fields et al. also demonstrated that TAT altered neuronal autophagy by modulating autophagosome fusion to the lysosome [27]. In this study, TAT exposure resulted in increased numbers of LC3-II puncta and autophagosomes in a neuronal-derived cell line *in vitro*. Similarly, *in vivo* studies in GFAP-TAT transgenic mice showed increased autophagosome accumulation in the neurons that was accompanied with altered LC3-II levels and neuron dysfunction. The findings from this study implicate that therapies targeting TAT-mediated autophagy alterations could mitigate neurodegeneration in HIV-1-infected individuals with HAND.

#### 4. Cocaine, HIV-1, and autophagy

Cocaine, one of most abused drugs, is known to lead to drug addiction. At the molecular level, cocaine binds to dopamine transporter to block dopamine uptake by the neurons, leading subsequently to elevated synaptic dopamine concentrations. Increased synaptic dopamine levels can, in turn, overstimulate the dopamine receptors located in the post-synaptic membrane resulting in enhanced neuronal excitability in the brain striatum [28]. Emerging evidence also demonstrates that cocaine-mediated microglial activation can contribute to the development of drug addiction [29]. In this study, it was shown that cocaine directly interacts with the Toll-like receptor 4-mediated pathway to activate microglia, resulting in enhanced microglial-neuronal cross talk and enhanced synaptic dopamine concentrations. Inhibition of microglial activation through pharmacological or genetic approaches was shown to block cocaine-mediated reward-related behavioral changes.

Compared to other abused drugs such as methamphetamine and morphine that have been implicated to dysregulate autophagy, reports on cocaine-mediated induction of autophagy in cells of the CNS are relatively scant. In a recent study, it was shown that [30] cocaine exposure resulted in induction of autophagy both in microglia and in astrocytes and also *in vivo* in mice administered cocaine. Cocaine exposure can lead to increased expression of various autophagy

markers such as Beclin-1, ATG5, and LC3-II in both a dose- and time-dependent manner. In this study, upstream activation of ER stress was shown to mediate induction of autophagy. It was shown that increased autophagy contributed to cocaine-mediated activation of microglia since pre-treatment of cells with wortmannin resulted in decreased expression and release of inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and CCL2). In another report, it was demonstrated that cocaine induced autophagic death in astrocytes, a process involving activation of sigma 1 receptor, PI3K, and mTOR pathway [31]. In another recent report, cocaine exposure of neurons was also shown to elicit autophagic cytotoxicity via a nitric oxide-GAPDH signaling cascade [32]. In this study, cocaine exposure markedly increased levels of LC3-II with a concomitant depletion of p62. Pharmacologic inhibition of autophagy protected neurons against cocaine-induced cell death.

Cocaine abuse is one of the comorbidities of HIV-1 infection. Cocaine abuse has been demonstrated to increase HIV-1 infection rate and to accelerate HAND pathogenesis [33, 34]. HIV-1-infected individuals abusing cocaine exhibited increased virus loads in the brain with associated neuroinflammation and neuronal injury compared to HIV-1-infected individuals that were drug naïve. Clinical evidence showed that cocaine use resulted in lack of virologic suppression and accelerated decline of CD4<sup>+</sup> T cells even among ART-adherent patients [35] which was consistent with findings that cocaine enhanced HIV-1 replication, as demonstrated in multiple *in vitro* and *in vivo* studies [36, 37]. In addition to its effects on virus replication, cocaine has also been shown to disrupt the integrity of brain-blood barrier, resulting in enhanced macrophage/monocyte influx into the brain, leading to increased neuroinflammation [38]. Additionally, cocaine has also been shown to potentiate toxicity of HIV-1 proteins such as gp120 in the brain [39]. Similar to gp120, cocaine and TAT exerted a synergistic neurotoxic effect in rat primary hippocampal neurons [40]. In these cells, cocaine exposure exacerbated TAT-induced mitochondrial depolarization and generation of intracellular ROS [40]. Cocaine can thus modulate the activation status of microglia and astrocytes through multiple mechanisms including ROS, ER stress, and autophagic pathways [30, 41].

## 5. Methamphetamine (METH), HIV-1, and autophagy

METH is a psychostimulant drug which is extensively abused for its stimulant, euphoric, empathogenic, and hallucinogenic properties [42]. METH predominantly disrupts the monoamine neurotransmitter system of the brain leading to persistent damage in the serotonin and dopamine neurotransmission, a mechanism also accountable for the motor deficiencies observed in Parkinson's disease [43]. While METH-mediated impairment in neurotransmission results in nigrostriatal denervation, accumulation of ubiquitin-positive neuronal inclusions, and striatal dopamine loss resulting in long-term neurotoxicity [42], METH does not affect dopaminergic neurons in the substantia nigra pars compacta. Although nigral cell bodies are largely preserved following exposure to METH, cytoplasmic features do reveal the presence of autophagic-like vacuolization and accumulation of  $\alpha$ -synuclein, ubiquitin, and parkin-positive inclusion-like bodies [44], as is the feature of several neurodegenerative diseases [45]. Chronic use of METH is closely coupled with cognitive deficits ranging from

impaired impulse control, attentional problems, working memory, and decision making to motor coordination, including inhibitory control [46]. Apart from this, chronic METH abuse also leads to a limited, but persistent, loss of dopamine and serotonin transporters in the striatum, cortex, and hippocampal areas. METH abuse also induces basal ganglia-mediated behavioral deficits, which is secondary to compromised dopamine neurotransmission in the moderately denervated striatum [47].

Emerging studies also correlate the use of METH with the activation of autophagy in both the CNS and periphery [48]. The effects of METH on the autophagic pathway are likely to depend on the superfluous free radicals and oxidative species generated within the cells. These effects become predominant within the dopaminergic neurons, which is likely due to the specificity of METH for dopamine targets and the ability of dopamine to self-oxidize and produce free radicals [49]. Indeed, the mechanisms of action of METH involve release of cytosolic dopamine and are based on various molecular targets such as the dopamine transporter, the vesicular monoamine transporter-2, and monoamine oxidase, which are involved in the uptake, storage, and release of dopamine [50]. By acting on the dopamine vesicular storage, METH interrupts the physiological gradient, with the diffusion of large amount of dopamine into the cytosol, where the blockage of monoamine oxidase type A abrogates its physiological metabolism, leaving the cytosolic dopamine vulnerable to self-oxidize to form dopamine-quinones and cysteinyl aggregates, thereby promoting neurotoxicity [49].

In addition to its direct neurotoxic effects, the dysfunction of blood-brain barrier (BBB) is also a feature of METH-induced neurotoxicity [51]. METH has been shown to induce the impairment of GLUT1 at the brain endothelium thereby contributing the energy-associated disruption of tight junction assembly and loss of BBB integrity [52]. Moreover, METH acts directly on cultured rat brain microvascular endothelial cells to compromise the BBB via the involvement of eNOS/NO-mediated transcytosis [51]. Additionally, METH mediates a transient increase in the permeability of the BBB in the hippocampus compared with the frontal cortex and striatum, via alterations in the expression levels of tight junction proteins and matrix metalloproteinase-9 [53]. Low doses of METH (1  $\mu$ M) have been shown to induce endothelial cell barrier dysfunction, thereby underscoring the role of METH in BBB compromise [51]. Interestingly, exposure of low doses of METH also induces induction of autophagy in two dopaminergic neuronal-derived cell lines such as the rat pheochromocytoma PC12 and the human neuroblastoma SH-SY5Y through the phosphatidylinositol 3-kinase III signaling. In contrast, caspase-dependent neuronal cell death involves inhibition of the autophagy maturation process despite the aggregation of  $\alpha$ -synuclein and damaged mitochondria in the cytoplasm of METH-exposed cells [44]. Similarly, METH exposure also leads to the induction of the autophagic sequestering process (by inducing LC3-II expression) in human dopaminergic SK-N-SH cell line by ameliorating the mTOR activity on its downstream phosphorylation of the target eukaryotic translation initiation factor 4E-binding protein 1, eventually resulting in decreased cell viability [54]. METH exposure of SK-N-SH cells also induces autophagy by blocking both the dissociation of the Bcl-2/Beclin-1 complex and upstream activation of c-Jun N-terminal kinase 1 signaling [55]. Acute METH exposure also has been shown to induce autophagy as an early protective response. Chronic METH exposure on the

other hand exacerbates the progression of autophagic flux leading, in turn, to apoptotic cell death in primary human brain microvascular endothelial cells and human umbilical vein endothelial cells, through the Kappa opioid receptor [56]. Recent investigations have also identified the potentiation of METH-mediated neurotoxicity by caffeine through inhibition of autophagy via the protein kinase A activation pathway and via enhancement of LC3-II phosphorylation, thereby abrogating incorporation of LC3-II into the autophagosomes [57]. In cardiomyocytes and dopaminergic neurons, METH exposure also stimulates the damage-inducible transcript 4 expression leading to induction of autophagy and apoptosis [58, 59].

METH use has been associated with higher risk-taking behaviors that set drug abusers at a higher risk of exposure to infections such as HIV-1 and hepatitis C, each, in turn, contributing eventually to CNS dysfunction. METH abuse has also been shown to accelerate the onset and severity of HAND. Neurotoxic consequences of METH abuse and HIV-1 infection include brain hyperthermia, release of inflammatory cytokines and reactive oxygen species, excitotoxicity, and astrogliosis [60]. METH abuse in combination with HIV-1 infection leads to notable variations in the functioning of dopaminergic neurons. Role of HIV-1 TAT protein that is actively released by infected glial and lymphoid cells has been well documented in the pathogenesis of HAND [61–63]. Intrastriatal administrations of HIV-1 TAT combined with gp120 protein have been shown to damage both efferent and afferent neurons in the striatum [64], including the nigrostriatal DA neurons [65], likely underlie the motor abnormalities observed in HIV-1-infected individuals. Co-exposure of HIV-1 TAT and METH cross-amplifies their deleterious cellular effects through oxidative stress-mediated inflammatory mediators such as TNF- $\alpha$ , IL- $\beta$ , and ICAM-1 in distinct regions of the mice brain, with implications for CNS complications in HIV-1-infected individuals abusing drugs. Additionally, mice administered HIV-1 TAT and METH were shown to exhibit enhanced DNA-binding activities of transcription factors such as NF- $\kappa$ B, AP-1, and CREB in the frontal cortex and hippocampal regions compared with mice administered either HIV-1 TAT or METH alone [60]. These findings likely suggest that HIV-1 TAT and METH synergistically decrease striatal dopamine release and content, which likely leads to increased risk for basal ganglia dysfunction and cognitive impairment in METH abusers that are infected with HIV-1 [66].

Interestingly, METH abuse is closely associated with higher viral loads in ART-receiving HIV-1-positive individuals [67]. The combination of METH and HIV-1 leads to increased neurocognitive deficits and neuropathology compared with the either agent alone [68]. The potential mechanistic interactions of virus, antiviral treatment, and the psychostimulant drug, however, remain largely unknown. Furthermore, METH-mediated dopamine accumulation in the synapse also increases the levels of free radicals in the neurons, thereby promoting protein damage as well as protein dysfunction, leading to upregulation of autophagy [48]. It has also been shown that exposure of neurons to varying combinations of cART and METH, along with HIV-1-envelope gp120, compromised cellular ATP homeostasis in association with activation of both AMP-activated protein kinase (AMPK) and autophagy [68]. Overall, METH-mediated neurotoxicity is mediated by induction of a specific cellular pathway that is activated when dopamine is not effectively sequestered in the synaptic vesicles, thereby producing oxidative stress, autophagy, and eventually neurite degeneration.

## 6. Morphine, HIV-1, and autophagy

Opiates are the most potent and popular compounds known to control pain and are also among the most common drugs of abuse. Heroin is one such highly addictive, illegal opioid drug. Since heroin is converted to morphine in the brain, morphine has been the preferred opiate of choice in most studies. Morphine is a natural opiate that has a clinically widespread use for pain management in cancer patients. Despite its beneficial effects, chronic use of opiates elicits adverse side effects, such as memory impairment, tolerance, dependence, drug addiction, and neural injury [69]. Morphine exposure also significantly alters the immune system by modifying the functions of a range of immune cells such as phagocytes, T cells, and dendritic cells [70, 71]. Morphine induces cellular impairment via inhibition of the central cholinergic system, altered expression of  $\mu$ -opioid receptor, attenuation of long-term potentiation in the hippocampus caused by accumulation of extracellular adenosine [72], increased glucocorticoid concentration in plasma [73], and inhibition of nitric oxide synthesis in the brain [74].

Morphine induces autophagy through sustained activation of  $\mu$ -opioid receptor in rat hippocampal neurons and in neuroblastoma SH-SY5Y cells resulting in neuronal injury [75]. Morphine-mediated induction of autophagy in these cells is operated primarily by PTX-sensitive G protein-coupled receptor signaling. Following its binding to  $\mu$ -opioid receptor, there is an increase in Beclin-1 protein levels and a significant decrease in the association of Beclin-1 with Bcl-2 leading, in turn, to dissociation of Beclin-1 from its pro-autophagic events. It has been reported that Bcl-2 overexpression remarkably impedes morphine-mediated autophagy induction and that genetic silencing of Beclin-1 or another autophagy marker ATG5 inhibits morphine-mediated autophagy. Long-term exposure of morphine has been shown to stimulate neuronal cell death, an effect that is exacerbated by genetic silencing of Beclin-1. Taken together, this study for the first time identified key roles of Beclin-1 and ATG5 in morphine-mediated induction of neuronal injury [75].

In another study, long-term morphine exposure was shown to induce autophagy in the superficial layer of the spinal cord through upregulation of Beclin-1, LC3-II, and cathepsin B in the GABAergic interneurons, resulting in the development of antinociceptive tolerance. Blockade of either cathepsin B or autophagy notably suppressed morphine-mediated antinociceptive tolerance [76]. Morphine abuse was also closely associated with decreased mitochondrial DNA copy number both in the hippocampus and in peripheral blood and was linked to drug addiction. These findings were also corroborated in heroin addicts [77]. Morphine exposure also has been shown to potentiate LPS-induced autophagy initiation and block the fusion of autophagosomes in macrophages, leading to defective bacterial clearance and increased bacterial load via TLR4-dependent and TLR4-independent pathways. Morphine exposure thus increases the susceptibility to infection as well as prevalence of persistent infection in drug addicts [78]. Morphine also increases autophagic flux both in the hippocampal CA1 neurons and in microglia, leading to increased neuronal cell death in the CA1 and CA3 regions and escalated inflammation in the hippocampal microglia, ultimately resulting in morphine-mediated spatial memory impairment [79].

Opiates abuse and HIV-1 are interlinked epidemics, and opiates such as heroin exacerbate the neuropathogenesis of HIV-1 with rapid disease progression [80]. Long-term opiate abuse in the pre-cART era was demonstrated to be directly associated with increased progression to HIV and exacerbated neuropathology in patients on cART [81]. Morphine exacerbates HIV-1 toxicity through distinct pathways in neurons and in glia, primarily through the  $\mu$ -opioid receptors. Acute exposure of morphine leads to increased HIV-1 replication in the infected microglia [82]. Morphine potentiates the deleterious effects of HIV-1 TAT via dysregulation of intracellular calcium homeostasis, leading to decreased buffering of extracellular glutamate in the astrocytes. This dysfunction in turn leads to decreased excitotoxicity threshold of neurons, resulting in increased production of reactive species and proinflammatory mediators, which ultimately damage the neurons [83]. Morphine exposure has also been shown to enhance both HIV-1 replication and inflammatory response through the Beclin-1-independent mechanism in microglial cells, implicating the connections between autophagy and HIV-1 pathogenesis [23]. In another study, chronic morphine exposure of HIV-1-infected human monocyte-derived macrophages led to significant alterations in the secretion of IL-6 and monocyte chemoattractant protein 2, thereby suggesting enhanced CNS inflammation in HIV-1-infected opiate abusers [84].

## 7. Conclusion

Overall, this chapter sheds light on HIV-1, drugs of abuse such as cocaine, methamphetamine, and morphine-mediated autophagy primarily in the CNS and periphery. Such effects could underlie the synergistic effects of HIV-1 and abused drugs in exacerbating symptoms of HAND. Further studies, however, are warranted to unravel the mechanistic roles of autophagy in CNS cells and periphery in HIV-1-infected individuals with a history of drug addiction. Primarily, how autophagy might be involved in these cells and its relative contribution to immunopathogenesis, particularly HAND, has yet to be determined. Investigating autophagy in various cell types involved in the pathogenesis of HIV-1 infection in the context of drug abuse could provide a basis for future development of novel therapeutic strategies aimed at treating HIV-1-infected individuals that abuse drugs.

### Conflict of interests

There are no conflicts of interest for any of the authors.

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