Blood-brain barrier and neuro-AIDS

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Abstract. Neuro-AIDS is becoming a major health problem among AIDS patients who experience improved survival under combined antiretroviral therapy (cART). Neuronal injury and loss are the critical issues of neuro-AIDS that need the entry of HIV into the central nervous system (CNS) via peripheral infected monocyte/macrophage carriers or viral direct penetration of blood-brain barrier (BBB). The mechanisms of HIV enhancing BBB permeability and entering CNS and the effect of drug abuse in HIV traffic across BBB are discussed. In addition, the current anti-HIV drugs, although they are effective in reducing plasma viral level, cannot eradicate the viruses completely from CNS. The possible mechanism of BBB hindrance and anti-HIV drug efflux by transport proteins, and general methods used to deliver antiretroviral drugs into brain are also discussed.

Key words: AIDS, Antiretroviral therapy, Blood-brain barrier, HIV, Nanomaterial.

Introduction

Approximately 33.3 million people worldwide are infected by human immunodeficiency virus (HIV), and nearly three million people become newly infected every year (UNAIDS Global Report 2010, www.unaids.org). It is well known that HIV has devastating effect on human immune system to result in acquired immunodeficiency syndrome (AIDS) with the characteristics of severe and unusual central nervous system (CNS) infection as the opportunistic infection (OI)¹. Although the survival of people infected with HIV has been improved due to the application of increasingly powerful and highly active antiretroviral agents including HIV protease inhibitors (PIs), nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and viral entry inhibitors, OI in CNS remains a serious issue worldwide²,³. In addition, neuro-AIDS is becoming a major health problem among AIDS patients and long-term HIV survivors in the era of highly active antiretroviral therapy (HAART). These problems include systemically well-controlled infection, memory problems and slowness, and difficulties in concentration, planning and multitasking⁴,⁵. The entry of HIV into the CNS is necessary for the occurrence and development of neuro-AIDS.

The structure and function of blood-brain barrier

CNS is one of the most protected organ systems in body, which is protected by blood-brain barrier (BBB) as a highly selective permeability barrier for separating circulating blood from brain extracellular fluid in CNS and maintaining the homeostasis of brain microenvironment necessary for stable and coordinated activity of neurons. The BBB is formed by brain microvascular endothelial cells (BMVECs), which are connected by tight junction (TJ) with an extremely high electrical resistance of at least 1000 Ωcm⁻², and a thick basement membrane and astrocytic endfeet⁷,⁸. The balance among matrix metalloproteinase 9 (MMP-9) and its natural inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), is important in maintaining the integrity of basement membrane⁹. BBB acts as a physical barrier because complex TJs between adjacent endothelial cells force molecular traffic to take a transcellular route. The presence of specific transport systems on the luminal and abluminal membrane regulates trans-cellular traffic, thus providing a selective transport barrier including both uptake mechanisms (e.g. GLUT-1 glucose carrier and L1 amino acid transporter) and efflux transporters (e.g. P-glycoprotein, P-gp). A combination of intracellular and extracellular enzymes allows BBB to serve as a metabolic barrier¹⁰.
BBB facilitates the entry of required nutrients into brain, and excludes or effluxes potentially harmful compounds, which will be benefit for separating the pools of neurotransmitters and neuroactive agents that act centrally and peripherally, and regulating the ionic microenvironment of neurons\textsuperscript{11,12}. Moreover, BMVECs can inhibit microbial invasion by immunological mechanisms. BMVECs can express functional toll-like receptor 3 (TLR3) that can be activated by polyinosinic-polycytidylic acid (PolyI:C), thus resulting in the induction of endogenous interferon-\(\beta\) (IFN-\(\beta\)) and IFN-\(\lambda\). The activation of TLR3 in BMVECs can also induce the phosphorylation of interferon regulatory transcription factor 3 (IRF3) and IRF7, the key regulators of IFN signaling pathway\textsuperscript{11,13}. Some parasites, bacteria, and viruses have, however, developed various CNS invasion strategies, and can bypass the brain barriers. As for viruses, these strategies include transport along neural pathways, transcytosis, brain endothelial cell infection, BBB breaching, and infected-leukocyte passage. Furthermore, neurotropic viruses can alter BBB functions, thus compromising CNS homeostasis\textsuperscript{12,14}. Similarly, HIV can change BBB permeability and enter brain by multiple complex mechanisms during the early stage of initial infection, thus resulting in a complicated array of diverse neurological dysfunctions defined as neuro-AIDS\textsuperscript{15}. Once HIV enters CNS, it cannot be completely cleared by HAART since BBB can prevent antiretroviral agents from free entry into brain, which will form a CNS HIV reservoir\textsuperscript{16}. How does HIV traffic through blood-brain barrier?

Neuronal injury and loss are the critical issues of neurological decline and HIV-associated neuropathology. HIV does not productively infect neurons due to the lack of CD4 receptor on the surface of neurons, although high levels of HIV DNA in peripheral blood mononuclear cells or cerebrospinal fluid (CSF) is correlated with the prevalence of neuro-AIDS\textsuperscript{15}. HIV-associated neuropathology is indirectly caused by viral proteins released from infected cells in CNS or host-derived inflammatory cytokines and chemokines released from infected and/or activated macrophages/microglial cells\textsuperscript{18,19}. Thus, it is necessary for the incidence of neuro-AIDS that HIV traffics through BBB and enters CNS.

**Monocyte/macrophage plays as the vehicle of delivering HIV through blood-brain barrier**

HIV can enter CNS within 2 weeks after infection and the earlier HIV enters the CNS the more difficult it might be to remove with antiretroviral therapy\textsuperscript{20}. The entry of HIV into CNS depends on peripheral infected monocyte/macrophage carriers or viral direct penetration of BBB that needs the high permeability of BBB\textsuperscript{21-25}. Particularly, the mature CD14(+)CD16(+) monocytes bring viruses across BBB into CNS parenchyma in response to chemotactic signals. It needs the homotypic and heterotypic interactions of junction proteins that are located on monocytes and BMVECs when the mature CD14(+)CD16(+) monocytes transmigrate across the barrier\textsuperscript{26-28}. Notably, CD4\(^+\)CD25\(^-\) regulatory T cells (Treg) readily migrating across BBB can transform HIV-1 infected macrophages from a neurotoxic phenotype to a neuroprotective phenotype\textsuperscript{29}. Interleukin (IL)-6, IL-8, and gp120 can increase monocyte adhesion and migration across in vitro BBB models. Monocyte chemotactic protein-1 (MCP-1) can affect the migration of macrophages through the regulation of voltage-gated K\(^+\) channels\textsuperscript{30}. The disruption of tight junction facilitates HIV across blood-brain barrier

The disruption of TJJs between BMVECs is mediated through the activation of focal adhesion kinase (FAK) and the disruption of actin cytoskeleton and matrix metalloproteinase activity\textsuperscript{31,32}. Specific TJ proteins, such as junction adhesion molecule-A (JAM)-A, occludin, and zonula occludens (ZO)-1, can localize not only at the cell-cell borders but also present in the nuclei. Rho signaling and cyclic adenosine monophosphate (cAMP) response element-binding (CREB) protein can modulate nuclear localization of ZO-1 and maintain the integrity of endothelial monolayer\textsuperscript{33}. HIV-infected monocytes decrease the expression of TJ proteins in BMVECs. The overexpression of peroxisome proliferator-activated receptor alpha or gamma (PPAR-a or PPAR-b) can attenuate HIV-mediated dysregulation of TJ proteins and inhibit the overexpression of HIV-induced MMP-9 in brain endothelial cells\textsuperscript{34,35}. The dysfunction of brain endothelial cells enhances the permeability of blood-brain barrier

HIV-1 and viral proteins, in addition to cellular mediators released from infected and uninfected cells, participate in astrocytic and neuronal dysreg-
ulation, thereby leading to neurological disorders. The molecular architecture of viral regulatory components including long terminal repeat (LTR) genes encoding viral proteins Tat, Vpr and Nef as well as the envelope gene encoding gp120 and gp41 has been implicated in indirect mechanisms of neuronal injury. HIV-1 and viral proteins can also disrupt BBB by interacting with cerebral cells including BMVECs via CD-4-receptors, CCR-5- and CXCR-4-coreceptors located at the surface of these cells. HIV can use mannose-6 phosphate receptor (M6PR) to cross BBB. Transport is dependent on cyclic adenosine monophosphate (cAMP) and calcium. Oxidative stress participates in HIV-induced BBB breakdown due to the large amount of mitochondria in cerebrovascular endothelial cells. Lipopolysaccharide (LPS) present in the plasma, CSF of HIV infected, and cognitively impaired individuals. Tat can induce BBB permeability in a CD40L-dependent manner. This permeability of BBB is found to be the result of aberrant platelet activation induced by Tat. The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) can induce the release of circulating sCD40L in HIV-infected individuals via the activation of glycogen synthase kinase 3 beta in platelets. Tat disrupts BBB integrity, at least in part by decreasing the production of occludins and miR-101 and peroxisome proliferator-activated receptor (PPAR) can regulate barrier permeability in BMVECs exposed to the Tat protein. Tat can up-regulate the expression of MMP-9 via MAPK-NF-kappaB-dependent mechanism and Tat-induced TNF-alpha production in astrocytes. Furthermore, crosstalk between signal transducers and activators of transcription 1 (STAT1), mitogen extracellular kinase (MEK), and phosphatidylinositol-3-kinases (PI3K) pathways is observed in gp120-induced BBB dysfunction. Acute and chronic exposure to gp120 can disrupt BBB via direct toxicity to brain endothelial cells including the activation of MMP and N-methyl-D-aspartate receptor 1 (NMDAR-1) and the degradation of vascular basement membrane and vascular TJs. Tissue destruction by MMPs is regulated by their endogenous tissue inhibitors (TIMPs). TIMPs can prevent excessive MMP-related degradation of extracellular matrix components. Paradoxically, gp120 can up-regulate TIMP-1 and TIMP-2 in CNS.

**Astrocyte and brain pericyte become the accomplices to exaggerate the permeability of blood-brain barrier**

Astrocyte, a composition of BBB, can be infect with HIV by contacting with monocytes/macrophages, and activated astrocytes can exaggerate monocyte recruitment into brain via MCP-1. A few HIV-infected astrocytes disrupt BBB integrity through gap junction as blocking these channels to protect BBB from HIV-infected astrocyte-mediated compromise, thus resulting in endothelial apoptosis, misguided astrocyte end-feet, and dys-regulation of lipoxygenase/cyclooxygenase, Calcium-activated potassium channels (BK) and adenosine triphosphate (ATP) receptor activation within astrocytes. Gene array analysis reveals an impressive increase in the transcription of the gene for CCL2/MCP-1 chemokine in HIV-1-infected astrocytes, which is a consequence of HIV-1-induced enhancement of membrane-associated tumor necrosis factor-alpha (TNF-α) in macrophages and correlated with the increased levels of nuclear factor kappa B activation in astrocytes. Human brain pericytes express the major HIV-1 receptor such as CD4 and co-receptors such as CXCR4 and CCR5. Pericytes are susceptible to infection with both X4-tropic NL4-3 and R5-tropic JR-CSF HIV-1 strains. Moreover, HIV-1 infection of pericytes can result in compromised integrity of BBB as an in vitro model.

**Drug abuse exacerbates HIV-associated BBB dysfunction**

Drug abuse and HIV infection are interlinked. Both laboratory-based and epidemiological studies strongly indicate that drug abuse may exacer-
bate HIV disease progression and increase mortality and morbidity of these patients. The effect of HIV infection and the application of intravenous drugs may be additive in increasing TNF-a level that can disrupt BBB. Cocaine, methamphetamine (METH) and morphine that are commonly used in patients infected with HIV have been demonstrated to exacerbate the neurotoxic effect of gp120 by increasing oxidative stress in CNS and the permeability of BBB. Morphine can induce the expression of dendritic cell markers including CD11c, macDC-SIGN and CD83 in brain parenchyma of SIV-infected macaques. In both preclinical and clinical studies, psychostimulants including METH, N-Methyl-3,4-methylenedioxyamphetamine (MDMA), cocaine and nicotine can produce BBB dysfunction through changing the expression and conformation of TJ proteins, increasing glial and astrocytes activation, improving enzyme activation associated with BBB cytoskeleton remodeling, and inducing neuroinflammatory pathways. METH has been demonstrated to increase viral load in CNS of SIV-infected macaques and exhibit detrimental effects on BBB, thus resulting in the potential to increase the probability of CNS infection by HIV. MMP level is substantially increased in the setting of HIV infection with METH abuse. Elevated MMP level in turn can affect the integrity of BBB. Elevated levels of MMPs can also contribute to the microglial activation and neuronal and synaptic injury. Cocaine exposure can lead to leakage of BBB that manifests as enhanced transmigration of leukocytes/monocytes into CNS. HIV-associated increase in monocyte adhesion and trafficking is exacerbated by cocaine abuse via cocaine-mediated induction of activated leukocyte cell adhesion molecule (ALCAM) as a mediator of increased monocyte adhesion/transmigration into CNS. Platelet-derived growth factor (PDGF) has been implicated in several pathologic conditions, specifically attributable to its potent mitogenic effects. Cocaine-mediated induction of PDGF-BB in human BMVECs through binding to its cognate receptor and activating mitogen-activated protein kinases and Egr-1 pathways, thus resulting in the increased permeability of the endothelial barrier. Notch1 activation is involved in cocaine-mediated regulation of PDGF-B expression. Cannabinoids with anti-inflammatory properties can cross BBB to target specific receptors. Cannabinoid agonists can inhibit HIV-induced calcium influx by substance P and CB(2) receptor (CB2R), and significantly decrease the permeability of BMVECs as well as prevent the down-regulation of TJ proteins such as ZO-1, claudin-5, and JAM-1 in human BMVECs. Furthermore, cannabinoid agonists can inhibit the transmigration of human monocytes across BBB and block BBB permeability in vivo.

Nicotine can also cause the dysfunction of BMVECs. Nicotine and gp120 are able to significantly increase the serum levels of ubiquitin C-terminal hydrolase 1 (UCHL1), a new BBB marker in mice, which are correlated with the change of it in circulating brain microvascular endothelial cells (eBMECs) and endothelial progenitor cells (EPCs). Furthermore, the integrity of BBB is disrupted after concurrent administration of chronic nicotine and protease inhibitors because nicotine and protease inhibitors can cause an additive oxidative stress burden in endothelial cells and multiple efflux transporters. BBB endothelial dysfunction is correlated with a decrease in Notch-4, a primary protein, involved in maintaining the stability of BBB endothelium, and ZO-1 expression. Apart from oxidative stress, the overproduction of pro-inflammatory factors, glutamate-associated neurotoxicity, HIV-1 and alcohol-mediated neurodegeneration are also correlated with the impairment of BBB.

The current status of antiretroviral therapy in CNS

The development of antiretroviral drugs over the past couple of decades has been commendable owing to the identification of several new targets within the overall HIV replication cycle. Even these antiretroviral agents with poor penetration into CNS, early antiretroviral treatment (ART) can prevent CNS dysfunction by decreasing brain viral load. But longer treatment may be required to completely resolve encephalitic lesions and microglial activation.

The challenge of anti-HIV in CNS

ART can remarkably reduce the prevalence of neurologic deficits for the majority of HIV-infected patients, but some patients such as children do not experience these benefits. Moreover, complete control over HIV/AIDS is not achieved yet because the current anti-HIV drugs, although they are effective in reducing plasma viral level, cannot eradicate the viruses completely from the body. In addition, it is also due to the insufficient accumulation of most anti-HIV drugs in certain cellular and anatomical reservoirs including CNS.
sufficient delivery of anti-HIV drugs to CNS is also attributed to their low permeability across BBB\(^{60,91}\). Moreover, some PIs may not penetrate into CNS at the therapeutic concentrations\(^{92}\). Hence, low and sustained viral replication within CNS continues and HIV-associated neurocognitive disorders occur even during prolonged CNS-targeted antiretroviral therapy\(^{93,94}\). Furthermore, some ART combinations are able to cross BBB, but they can also cause important CNS-related side effects\(^{95,96}\). The application of well-tolerated ART medications that are able to penetrate BBB can realize particular promise, as these agents may increase viral suppression in the parenchyma and reduce neurocognitive dysfunction. However, ART restricts the synthesis of infectious viruses but does not curtail HIV-1 transcription and translation from either the integrated or unintegrated viral genomes in infected cells. All treated patients with full viral suppression actually have low-level viremia. Thus, new therapeutic agents are needed to curtail HIV-1 transcription and residual viruses.

**Transport proteins of blood-brain barrier efflux antiretroviral drugs**

Drug transport in CNS can be highly regulated by the expression of numerous influx and efflux transport proteins not only at BBB and blood-cerebrospinal fluid barrier but also in brain parenchymal cellular compartments (i.e., astrocytes, microglia, and neurons). In particular, the members of ATP-binding cassette (ABC) membrane associated with transporter superfamily and solute carrier (SLC) family are known to be involved in the efflux and/or influx of drugs, respectively\(^{97,98}\). As a result, the changes in the functional expression of these transporters can alter the disposition and distribution of drugs in brain. HIV can up-regulate ABC superfamily member and develop drug resistance in endothelial cells of human brain via Rho signaling\(^{99}\). Moreover, antiretroviral therapy itself and/or pathological events (i.e., inflammation and oxidative stress) associated with viral infection may affect the functional expression of these transporters\(^{100}\). Thus, the CNS delivery of anti-HIV drugs is limited by blood-brain and blood-CSF interfaces due to a combination of restricted paracellular movement, powerful metabolic enzymes and numerous transporters including members of the ABC and SLC superfamilies. As a result, brain becomes a viral sanctuary site. This not only results in virological resistance, but also is associated with the development of complications such as HIV-associated dementia\(^{101}\). The ABC transporters expressed on human BMVECs (HBMVECs) can efflux HIV-1 protease inhibitors. Constitutive low expression of several ABC-transporters, such as multidrug-resistance gene 1 (MDR1 or P-gp) and multidrug resistance-associated proteins (MRPs), are documented in HBMVECs\(^{102}\). Notably, P-gp variant (3435C→T) has been reported to affect P-gp expression\(^{103}\). In addition, P-gp expression is regulated by ligand-activated nuclear receptors such as human pregnane X receptor (hPXR) and human constitutive androstane receptor (hCAR), and these receptors could represent potential pathways involved in P-gp induction by antiretroviral drugs. Amprenavir, atazanavir, darunavir, efavirenz, ritonavir, and lopinavir have been found to activate hPXR, whereas abacavir, efavirenz, and nevirapine have been found to activate hCAR. P-gp expression and function are significantly induced in hCMEC/D3 cells subjected to the treatment of these drugs at clinical concentrations in plasma\(^{104-106}\). Although it is recognized that inflammatory cytokines and exposure to xenobiotic drug substrates (e.g. PIs) can augment the expression of these transporters, whether concomitant exposure to viruses and antiretroviral drugs can increase drug-efflux functions in HBMVEC is still unknown. Therefore, MDR1 specific drug-efflux function can increase in HB-MVEC following co-exposure to HIV-1 and saquinavir (SQV), which can reduce the penetration of PIs into the HIV-1-infected brain reservoirs\(^{107,108}\). The glial cell compartment can act as a viral reservoir behind BBB. It provides an additional roadblock to effective pharmacological treatment via the expression of multiple drug efflux transporters, including P-gp. HIV/AIDS patients are frequently suffered from bacterial and viral co-infections, which can lead to the deregulation of glial cell function and the release of pro-inflammatory mediators including cytokines, chemokines, and nitric oxide\(^{109}\). The expression and function of multiple drug efflux transporters located at glial cells will be affected by inflammatory mediators.

**Strategy to deliver antiretroviral drugs into brain**

There is a high variability of antiretroviral drugs to reach the therapeutic concentration in cerebrospinal fluid, which is dependent on the characteristics of antiretroviral drugs (molecular weight, lipophilicity and protein-binding capability) and the binding capacity to substrate for efflux transporters\(^{110}\). Transporter kinetic measurements
show that large lipophilic drugs such as PIs have strong binding affinities to drug efflux transporters expressed at BBB, thereby preventing them from entering brain. However, when combined, the PIs with the highest binding affinity (i.e., boosting ritonavir) will occupy a large proportion of transporter binding sites and slow down the efflux rate of the co-administered PIs to facilitate the entry into brain. General methods can enhance drug delivery to brain. Various strategies like non-invasive methods, including drug manipulation encompassing transformation into lipophilic analogues, pro-drugs, chemical drug delivery, carrier-mediated drug delivery, receptor/vector mediated drug delivery and intranasal drug delivery, are exploited to deliver drugs to brain through olfactory and trigeminal neuronal pathways, and widely used. On the other hand, the invasive methods, primarily relying on the disruption of BBB integrity by osmotic or biochemical means, or direct intracranial drug delivery by intracerebroventricular, intracerebral or intrathecal administration after creating reversible openings in brain, are also recognized.

**Dimeric inhibitors of P-gp**

A series of dimeric inhibitors of P-gp are based on the nucleoside reverse transcriptase inhibitor and P-gp substrate, abacavir. These dimeric pro-drugs are designed to accomplish two purposes: inhibiting P-gp, the major drug efflux protein at BBB, by occupying two substrate-binding sites in the transporter; and pro-drug dimers that gain entry into endothelial cells at BBB will revert to their monomeric forms in the reducing environment of the cytosol due to the breakdown of the traceless tether, thus delivering abacavir for the therapy. The tether length of dimeric abacavir derivatives has a significant effect on the inhibition of P-gp drug efflux. Abacavir dimers can display potent inhibition of P-gp in two different cellular settings and revert to active abacavir in the reducing environment of HIV-infected T cells, thus leading to antiviral activity.

**Nanocarriers**

Nanocarriers including polymeric nanoparticles, liposomes, solid-lipid nanoparticles (SLN) and micelles can increase the local drug concentration gradients, facilitate drug transport into brain via endocytotic pathways and inhibit ABC transporters expressed at the barrier sites. Nanomaterials, as a result of their small size (in the order of many protein-lipid clusters routinely transported by cells) and their large surface area (as a scaffold for proteins to render nanoparticles as biological entities), offer a great promise for neuro-therapeutics. Alternatively, they can be optimized to affect their size, shape, and protein and lipid coatings to facilitate drug uptake, release and ingress across the barrier. The application of nanoparticles coated with polysorbate 80 or attached apolipoprotein E can ensure the delivery of drugs across BBB. Following conjugation with transferrin (Tf), saquinavir and amprenavir can permeate across biological barriers such as BBB via a receptor-mediated transport mechanism. A significant uptake of quantum rod QR-Tf-saquinavir or quantum dot QD-Tf-amprenavir by BMVECs, and a significant enhancement in transversing capability of these drugs across BBB, as well as a marked decrease in HIV-1 viral replication in peripheral blood mononuclear cells (PBMCs) are observed. A macrophage-carriage system for nanoformulated crystal PIs (atazanavir, ritonavir, indinavir, and efavirenz) can facilitate drug entry into brain. In addition, nano-NRTIs, nanocarriers for potential brain delivery of activated NRTIs are developed. Nanogel carriers are composed of poly(ethylene glycol) (PEG)- or pluronic-polyethyleneimine (PEI) biodegradable networks, star PEG-PEI or poly(amidoamine) dendrimer-PEI-PFG dendritic networks, and nanogels decorated with brain-targeting peptide molecules specifically binding to the apolipoprotein E receptor. Nano-NRTIs with a core-shell structure and decorated with brain-targeting peptides display the highest antiviral efficacy. Mitochondrial DNA depletion, a major cause of NRTI neurotoxicity, is reduced when compared with NRTIs at application of selected nano-NRTIs. Furthermore, an increase in the grafting quantity of CRM197 enhances the permeability coefficient of AZT across BBB and the uptake of AZT-loaded CRM197-grafted polybutylcyanoacrylate (PBCA) nanoparticles (NPs) (CRM197/PBCA NPs) by HBMECs.

**Magnetic-electric nanoparticle**

Inefficient cellular phosphorylation of NRTIs to their active nucleoside 5'-triphosphate (NTPs) forms is one of the limitations for HIV therapy. 3'-Azido-3'-deoxythymidine-5'-triphosphate (AZTTP) can be directly bound onto magnetic nanoparticles via ionic interaction. Magnetic-electric nanoparticles as field-controlled drug...
Carriers offer a unique capability of field-triggered release after crossing BBB via the modulation of P-gp and MRP localized on the luminal side of HBMECs\textsuperscript{132,133}. The apparent permeability of magnetic AZTTP liposomes is 3-fold higher than free AZTTP. The magnetic AZTTP liposomes are also efficiently taken up by monocytes and these magnetic monocytes show enhanced trans-endothelial migration when compared with normal/non-magnetic monocytes in the presence of an external magnetic field\textsuperscript{134}. Moreover, AZT is covalently attached to the recently reported sorbitol-G8 transporter, and the conjugate is found to target mitochondria in HeLa cells and readily cross BBB to gain the accessibility into mouse brain\textsuperscript{135}. Methylmethacrylate-sulfopropylmethacrylate (MMA-SPM) nanoparticles with grafted RMP-7 (RMP-7/MMA-SPM NPs) deliver stavudine (D4T), delavirdine (DLV), and saquinavir (SQV) across BBB. Smaller RMP-7/MMA-SPM NPs can yield a larger drug loading efficiency. The order of drug in the loading efficiency and in the particle uptake is D4T > DLV > SQV and D4T > SQV > DLV, respectively\textsuperscript{136}. Morphine exposure is known to induce apoptosis, downregulate cAMP response element-binding (CREB) expression and decrease dendritic branching and spine density in cultured cells. The magnetic nanoparticle (MNP)-based carriers bound to brain-derived neurotrophic factor are developed to treat opiate addiction, and protect neurotoxicity and synaptic density degeneration\textsuperscript{137}.

**Invasive methods and comorbid treatment**

Intranasal nanoemulsion is used for CNS targeting of saquinavir mesylate and found to have a high drug targeting efficiency\textsuperscript{143}. In addition, the improved treatment of comorbid medical conditions that are common in patients with HIV (e.g., HCV, liver failure and metabolic syndrome) is critical, as several of these conditions are known to have a significant effect on neural functions\textsuperscript{144}. HIV-1 may directly contribute to the accumulation amyloid beta at BBB. In addition, statins may protect against increased amyloid beta levels associated with HIV-1 infection in brain\textsuperscript{145,146}.

**Conclusions**

Neuro-AIDS is becoming a major health problem among AIDS patients who experience improved survival in the era of HAART. Neuronal injury and loss are the critical issues of neuro-AIDS that needs the entry of HIV into CNS and inflammatory initiation via peripheral infected monocyte/macrophage carrier or viral direct penetration of BBB. HIV can change BBB permeability and enter brain by multiple complex mechanisms during the early stage of initial infection resulting in a complicated array of diverse neurological dysfunctions. Drug abuse can exacerbate HIV-associated BBB dysfunction. The current anti-HIV drugs, although they are effective in reducing plasma viral level, cannot eradicate viruses completely from CNS because of the hindrance and transport protein efflux of BBB. Thus, general methods are used to deliver antiretroviral drugs into brain, including drug manipulation encompassing transformation into lipophilic analogues, pro-drugs, chemical drug delivery, carrier-mediated drug delivery, receptor/vector mediated drug delivery and intranasal drug delivery.
Acknowledgements

This work is supported by National Natural Science Foundation of China (81371399, 81571178 and 81272266) and Twelfth Key Science and Technology Five Year Plan of China (2012ZX10001-002, 2012ZX10001-003, 2012ZX10001-004 and 2012BAI15B08).

Conflict of interest

The authors declare that they have no conflict of interest.

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