European Review for Medical and Pharmacological Sciences 2015; 19: 4927-4939

Blood-brain barrier and neuro-AIDS

Y.-L. ZHANG[1], Y.-B. OUYANG[1], L.-G. LIU[2], D.-X. CHEN[1]

[1]STD/AIDS Research Center, Beijing Key Laboratory (No. BZ0089), Capital Medical University affiliated Beijing You An Hospital, Beijing Institute of Hepatology, Beijing, China.
[2]Department of Infectious Diseases, The third Hospital of Changzhou, Changzhou Institute of Hepatology, Changzhou City, Jiangsu Province, China.

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 worldwide[2,3]. 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 multitasking[4-6]. 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 endfeet[7,8]. 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 membrane[9]. 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 barrier[10].

Corresponding Author: Dexi Chen, Ph.D, MD; e-mail: dexi0963@yahoo.com
Longgen Liu, MD; e-mail: ssewllg@163.com

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[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-β (IFN-β) and IFN-λ. 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[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[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[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[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[17]. 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[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[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[21-25]. Particularly, the mature CD14(+)CD16(+) monocytes bring viruses across BBB into CNS parenchyma in response to chemotactic signals. It needs the homo- and heterotypic interactions of junction proteins that are located on monocytes and BMVECs when the mature CD14(+)CD16(+) monocytes transmigrate across the barrier[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[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[30].

The disruption of tight junction facilitates HIV across blood-brain barrier

The disruption of TJs between BMVECs is mediated through the activation of focal adhesion kinase (FAK) and the disruption of actin cytoskeleton and matrix metalloproteinase activity[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[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[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[36]. 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[37]. HIV can use mannose-6 phosphate receptor (M6PR) to cross BBB. Transport is depended on cyclic adenosine monophosphate (cAMP) and calcium[38]. Oxidative stress participates HIV-induced BBB breakdown due to the large amount of mitochondria in cerebrovascular endothelial cells[39]. Lipopolysaccharide (LPS) present in the plasma of patients with HIV-1 can enhance transcellular transport of HIV-1 across BBB through the activation of p38 mitogen-activated protein kinase (MAPK) signaling in BMVECs. The p44/42 and p38 MAPK signaling pathways can mediate the LPS-enhanced release of IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF). These cytokines, in turn, can act at the luminal surface of BMVECs to enhance the transcellular transport of HIV-1 independent of the actions on paracellular permeability[40]. Moreover, LPS can act at the luminal surface of brain endothelial cells to induce abluminal secretion and stimulate pericytes to release substances for enhancing the permeability of the BMVEC monolayer to HIV[41]. HIV-1-derived Tat protein variants contain a trans-membrane domain, which may make them cross BBB and reach brain[42]. Tat B disrupts BBB integrity to a greater extent when compared to Tat C and cocaine further differentially exacerbates BBB dysfunction[43]. The level of the inflammatory mediator, soluble CD40 ligand (sCD40L), is elevated 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[44]. 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[45]. Tat disrupts BBB integrity, at least in part by decreasing the production of occludins[46,47]. miR-101 and peroxisome proliferator-activated receptor (PPAR) can regulate barrier permeability in BMVECs exposed to the Tat protein[48,49]. Tat can up-regulate the expression of MMP-9 via MAPK-NF-kappaB-dependent mechanism and Tat-induced TNF-alpha production in astrocytes[50]. Furthermore, crosstalk between signal transducers and activators of transcription 1 (STAT1), mitogen extracellular kinase (MEK), and phosphatidylinositide 3-kinases (PI3K) pathways is observed in gp120-induced BBB dysfunction[51,52]. 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[53,54]. 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[55,56].

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[37]. 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[57]. 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-α (TNF-α) in macrophages and correlated with the increased levels of nuclear factor kappa B activation in astrocytes[58]. 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[59].

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[60]. The effect of HIV infection and the application of intravenous drugs may be additive in increasing TNF-a level that can disrupt BBB[61]. 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[62-65]. Morphine can induce the expression dendric cell markers including CD11c, macDC-SIGN and CD83 in brain parenchyma of SIV-infected macaques[66]. 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[67-69]. 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[70]. 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[71]. Cocaine exposure can lead to leakage of BBB that manifests as enhanced transmigration of leukocytes/monocytes into CNS[72]. 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[73]. 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 σ receptor and activating mitogen-activated protein kinases and Egr-1 pathways, thus resulting in the increased permeability of the endothelial barrier[74,75]. Notch1 activation is involved in cocaine-mediated regulation of PDGF-B expression[76]. 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*[77-79].

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 (cBMECs) and endothelial progenitor cells (EPCs)[80]. 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[81]. 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[82]. 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[83].

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[84]. But longer treatment may be required to completely resolve encephalitic lesions and microglial activation[85].

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[86,87]. 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[88,89]. In-

sufficient delivery of anti-HIV drugs to CNS is also attributed to their low permeability across BBB[90,91]. 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 HBMVEC 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[111]. 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[93,112-114]. 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[115].

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[116]. 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[117,118].

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[119]. 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[120]. 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[121,122]. The application of nanoparticles coated with polysorbate 80 or attached apolipoprotein E can ensure the delivery of drugs across BBB[123]. 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[124,125]. A macrophage-carriage system for nanoformulated crystal PIs (atazanavir, ritonavir, indinavir, and efavirenz) can facilitate drug entry into brain[126-128]. 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-polyethylenimine (PEI) biodegradable networks, star PEG-PEI or poly(amidoamine) dendrimer-PEI-PEG 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[129]. 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[130].

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[131]. Magneto-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[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[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[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[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[137].

Other non-invasive methods

According to previous demonstration, fullerene C (60) can cross BBB by hybridizing a biologically active moiety dyad, which provides a promising clue as a pharmacological therapy of neural disorders[138]. Two nonpeptidic PIs, GRL-04810 and GRL-05010, are synthesized by adding two fluorine atoms to their bis-THF moieties and confirmed to enhance their penetration across BBB[139]. Many antiviral drugs including zidovudine, acyclovir, tenofovir, lamivudine, and stavudine are Oat substrates. Besides any effects on BBB, specific inhibitors of Oat1 and Oat3 may alter CNS drug levels by blocking organic anion transporters in the choroid plexus[140]. Luteolin has antiviral activity in a latent HIV-1 reactivation model and can effectively ablate both clade-B- and -C -Tat-driven LTR transactivation although it does not reveal the effect on Tat expression and its sub-cellular localization141. Neurokinin-1 receptor (NK1R) antagonists interfere with the binding of neuropeptide substance P to NK1R and exhibit anti-HIV-1 activity. NK1R antagonists can effectively penetrate BBB to reduce the inflammatory response within brain and have equally high anti-HIV-1 activity against all major HIV-1 subtypes. NK1R antagonists can synergistically interact with PIs, but not NRTIs, NNRTIs or viral entry inhibitors[142].

Invasive methods and comorbid treatment

Intranasal nanoemulsion is used for CNS targeting of saquinavir mesylate and found to have a high drug targeting efficiency[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[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[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 informatory 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.

References

1. Young J, Psichogiou M, Meyer L, Ayayi S, Grabar S, Raffi F, Reiss P, Gazzard B, Sharland M, Gutierrez F, Obel N, Kirk O, Miro JM, Furrer H, Castagna A, De Wit S, Munoz J, Kjaer J, Grarup J, Chene G, Bucher H. CD4 cell count and the risk of AIDS or death in HIV-Infected adults on combination antiretroviral therapy with a suppressed viral load: a longitudinal cohort study from COHERE. PLoS Med 2012; 9: e1001194.
2. Tan IL, Smith BR, von Geldern G, Mateen FJ, McArthur JC. HIV-associated opportunistic infections of the CNS. Lancet Neurol 2012; 11: 605-617.
3. Riveiro-Barciela M, Falco V, Burgos J, Curran A, Van den Eynde E, Navarro J, Villar del Saz S, Ocana I, Ribera E, Crespo M, Pahissa A. Neurological opportunistic infections and neurological immune reconstitution syndrome: impact of one decade of highly active antiretroviral treatment in a tertiary hospital. HIV Med 2013; 14: 21-30.
4. Giunta B, Ehrhart J, Obregon DF, Lam L, Le L, Jin J, Fernandez F, Tan J, Shytle RD. Antiretroviral medications disrupt microglial phagocytosis of beta-amyloid and increase its production by neurons: implications for HIV-associated neurocognitive disorders. Mol Brain 2011; 4: 23.
5. Roberts TK, Buckner CM, Berman JW. Leukocyte transmigration across the blood-brain barrier: perspectives on neuroAIDS. Front Biosci (Landmark Ed) 2010; 15: 478-536.
6. Cysique LA, Vaida F, Letendre S, Gibson S, Cherner M, Woods SP, McCutchan JA, Heaton RK, Ellis RJ. Dynamics of cognitive change in impaired HIV-positive patients initiating antiretroviral therapy. Neurology 2009; 73: 342-348.
7. Verma AS, Singh UP, Dwivedi PD, Singh A. Contribution of CNS cells in NeuroAIDS. J Pharm Biomed Sci 2010; 2: 300-306.
8. Potschka H. Targeting the brain--surmounting or bypassing the blood-brain barrier. Handb Exp Pharmacol 2010; (197): 411-431.
9. Bonoiu A, Mahajan SD, Ye L, Kumar R, Ding H, Yong KT, Roy I, Aalinkeel R, Nair B, Reynolds JL, Sykes DE, Imperiale MA, Bergey EJ, Schwartz SA, Prasad PN. MMP-9 gene silencing by a quantum dot-siRNA nanoplex delivery to maintain the integrity of the blood brain barrier. Brain Res 2009; 1282: 142-155.
10. Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permeability. J Anat 2002; 200: 629-638.
11. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006; 7: 41-53.
12. Miller F, Afonso PV, Gessain A, Ceccaldi PE. Blood-brain barrier and retroviral infections. Virulence 2012; 3: 222-229.
13. Li J, Wang Y, Wang X, Ye L, Zhou Y, Persidsky Y, Ho W. Immune activation of human brain microvascular endothelial cells inhibits HIV replication in macrophages. Blood 2013; 121: 2934-2942.
14. Stamatovic SM, Keep RF, Andjelkovic AV. Brain endothelial cell-cell junctions: how to "open" the blood brain barrier. Curr Neuropharmacol 2008; 6: 179-192.
15. Hazleton JE, Berman JW, Eugenin EA. Novel mechanisms of central nervous system damage in HIV infection. HIV AIDS (Auckl) 2010; 2: 39-49.
16. Joska JA, Hoare J, Stein DJ, Flisher AJ. The neurobiology of HIV dementia: implications for practice in South Africa. Afr J Psychiatry (Johannesbg) 2011; 14: 17-22.
17. Kallianpur KJ, Kirk GR, Sailasuta N, Valcour V, Shiramizu B, Nakamoto BK, Shikuma C. Regional cortical thinning associated with detectable levels of HIV DNA. Cereb Cortex 2012; 22: 2065-2075.
18. Kovalevich J, Langford D. Neuronal toxicity in HIV CNS disease. Future Virol 2012; 7: 687-698.
19. Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. J Neuroimmune Pharmacol 2009; 4: 430-447.
20. Milush JM, Chen HL, Atteberry G, Sodora DL. Early detection of simian immunodeficiency virus in the central nervous system following oral administration to rhesus macaques. Front Immunol 2013; 4: 236.
21. Sharma D, Bhattacharya J. Cellular & molecular basis of HIV-associated neuropathogenesis. Indian J Med Res 2009; 129: 637-651.
22. Ivey NS, MacLean AG, Lackner AA. Acquired immunodeficiency syndrome and the blood-brain barrier. J Neurovirol 2009; 15: 111-122.
23. Strazza M, Pirrone V, Wigdahl B, Nonnemacher MR. Breaking down the barrier: the effects of HIV-1 on the blood-brain barrier. Brain Res 2011; 1399: 96-115.
24. Gras G, Kaul M. Molecular mechanisms of neuroinvasion by monocytes-macrophages in HIV-1 infection. Retrovirology 2010; 7: 30.
25. Waki K, Freed EO. Macrophages and Cell-Cell Spread of HIV-1. Viruses 2010; 2: 1603-1620.
26. Williams DW, Calderon TM, Lopez L, Carvallo-Torres L, Gaskill PJ, Eugenin EA, Morgello S, Berman JW. Mechanisms of HIV entry into the CNS: increased sensitivity of HIV infected CD14+CD16+ monocytes to CCL2 and key roles of CCR2, JAM-A, and ALCAM in diapedesis. PLoS One 2013; 8: e69270.
27. Williams DW, Eugenin EA, Calderon TM, Berman JW. Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. J Leukoc Biol 2012; 91: 401-415.

28. Buckner CM, Calderon TM, Willams DW, Belbin TJ, Berman JW. Characterization of monocyte maturation/differentiation that facilitates their transmigration across the blood-brain barrier and infection by HIV: implications for NeuroAIDS. Cell Immunol 2011; 267: 109-123.
29. Gong N, Liu J, Reynolds AD, Gorantla S, Mosley RL, Gendelman HE. Brain ingress of regulatory T cells in a murine model of HIV-1 encephalitis. J Neuroimmunol 2011; 230: 33-41.
30. Gendelman HE, Ding S, Gong N, Liu J, Ramirez SH, Persidsky Y, Mosley RL, Wang T, Volsky DJ, Xiong H. Monocyte chemotactic protein-1 regulates voltage-gated K+ channels and macrophage transmigration. J Neuroimmune Pharmacol 2009; 4: 47-59.
31. Ivey NS, Renner NA, Moroney-Rasmussen T, Mohan M, Redmann RK, Didier PJ, Alvarez X, Lackner AA, MacLean AG. Association of FAK activation with lentivirus-induced disruption of blood-brain barrier tight junction-associated ZO-1 protein organization. J Neurovirol 2009; 15: 312-323.
32. Spindler KR, Hsu TH. Viral disruption of the blood-brain barrier. Trends Microbiol 2012; 20: 282-290.
33. Zhong Y, Zhang B, Eum SY, Toborek M. HIV-1 Tat triggers nuclear localization of ZO-1 via Rho signaling and cAMP response element-binding protein activation. J Neurosci 2012; 32: 143-150.
34. Huang W, Eum SY, Andras IE, Hennig B, Toborek M. PPARalpha and PPARgamma attenuate HIV-induced dysregulation of tight junction proteins by modulations of matrix metalloproteinase and proteasome activities. FASEB J 2009; 23: 1596-1606.
35. Huang W, Andras IE, Rha GB, Hennig B, Toborek M. PPARalpha and PPARgamma protect against HIV-1-induced MMP-9 overexpression via caveolae-associated ERK and Akt signaling. FASEB J 2011; 25: 3979-3988.
36. Irish BP, Khan ZK, Jain P, Nonnemacher MR, Pirrone V, Rahman S, Rajagopalan N, Suchitra JB, Mostoller K, Wigdahl B. Molecular Mechanisms of Neurodegenerative Diseases Induced by Human Retroviruses: A Review. Am J Infect Dis 2009; 5: 231-258.
37. Beliakov NA, Medvedev SV, Trofimova TN, Rassokhin VV, Dement'eva NE, Shelomov SA. [Mechanisms of cerebral damage in patients with HIV-infection]. Vestn Ross Akad Med Nauk 2012; (9): 4-12.
38. Dohgu S, Ryerse JS, Robinson SM, Banks WA. Human immunodeficiency virus-1 uses the mannose-6-phosphate receptor to cross the blood-brain barrier. PLoS One 2012; 7: e39565.
39. Grammas P, Martinez J, Miller B. Cerebral microvascular endothelium and the pathogenesis of neurodegenerative diseases. Expert Rev Mol Med 2011; 13: e19.
40. Dohgu S, Fleegal-DeMotta MA, Banks WA. Lipopolysaccharide-enhanced transcellular transport of HIV-1 across the blood-brain barrier is mediated by luminal microvessel IL-6 and GM-CSF. J Neuroinflammation 2011; 8: 167.
41. Dohgu S, Banks WA. Brain pericytes increase the lipopolysaccharide-enhanced transcytosis of HIV-1 free virus across the in vitro blood-brain barrier: evidence for cytokine-mediated pericyte-endothelial cell crosstalk. Fluids Barriers CNS 2013; 10: 23.
42. Cooper I, Sasson K, Teichberg VI, Schnaider-Beeri M, Fridkin M, Shechter Y. Peptide derived from HIV-1 TAT protein destabilizes a monolayer of endothelial cells in an in vitro model of the blood-brain barrier and allows permeation of high molecular weight proteins. J Biol Chem 2012; 287: 44676-44683.
43. Gandhi N, Saiyed ZM, Napuri J, Samikkannu T, Reddy PV, Agudelo M, Khatavkar P, Saxena SK, Nair MP. Interactive role of human immunodeficiency virus type 1 (HIV-1) clade-specific Tat protein and cocaine in blood-brain barrier dysfunction: implications for HIV-1-associated neurocognitive disorder. J Neurovirol 2010; 16: 294-305.
44. Davidson DC, Hirschman MP, Sun A, Singh MV, Kasischke K, Maggirwar SB. Excess soluble CD40L contributes to blood brain barrier permeability in vivo: implications for HIV-associated neurocognitive disorders. PLoS One 2012; 7: e51793.
45. Davidson DC, Schifitto G, Maggirwar SB. Valproic acid inhibits the release of soluble CD40L induced by non-nucleoside reverse transcriptase inhibitors in human immunodeficiency virus infected individuals. PLoS One 2013; 8: e59950.
46. Xu R, Feng X, Xie X, Zhang J, Wu D, Xu L. HIV-1 Tat protein increases the permeability of brain endothelial cells by both inhibiting occludin expression and cleaving occludin via matrix metalloproteinase-9. Brain Res 2012; 1436: 13-19.
47. Andras IE, Toborek M. HIV-1-induced alterations of claudin-5 expression at the blood-brain barrier level. Methods Mol Biol 2011; 762: 355-370.
48. Mishra R, Singh SK. HIV-1 Tat C modulates expression of miRNA-101 to suppress VE-cadherin in human brain microvascular endothelial cells. J Neurosci 2013; 33: 5992-6000.
49. Huang W, Chen L, Zhang B, Park M, Toborek M. PPAR agonist-mediated protection against HIV Tat-induced cerebrovascular toxicity is enhanced in MMP-9-deficient mice. J Cereb Blood Flow Metab 2014; 34: 646-653.
50. Ju SM, Song HY, Lee JA, Lee SJ, Choi SY, Park J. Extracellular HIV-1 Tat up-regulates expression of matrix metalloproteinase-9 via a MAPK-NF-kappaB dependent pathway in human astrocytes. Exp Mol Med 2009; 41: 86-93.
51. Yang B, Akhter S, Chaudhuri A, Kanmogne GD. HIV-1 gp120 induces cytokine expression, leukocyte adhesion, and transmigration across the blood-brain barrier: modulatory effects of STAT1 signaling. Microvasc Res 2009; 77: 212-219.
52. Yang B, Singh S, Bressani R, Kanmogne GD. Crosstalk between STAT1 and PI3K/AKT signaling in HIV-1-induced blood-brain barrier dysfunction: role of CCR5 and implications for viral neuropathogenesis. J Neurosci Res 2010; 88: 3090-3101.
53. Louboutin JP, Reyes BA, Agrawal L, Maxwell CR, Van Bockstaele EJ, Strayer DS. Blood-brain barrier abnormalities caused by exposure to HIV-1 gp120--protection by gene delivery of antioxidant enzymes. Neurobiol Dis 2010; 38: 313-325.
54. Louboutin JP, Strayer DS. Blood-brain barrier abnormalities caused by HIV-1 gp120: mechanistic and therapeutic implications. ScientificWorldJournal 2012; 2012: 482575.

55. Louboutin JP, Agrawal L, Reyes BA, Van Bockstaele EJ, Strayer DS. HIV-1 gp120-induced injury to the blood-brain barrier: role of metalloproteinases 2 and 9 and relationship to oxidative stress. J Neuropathol Exp Neurol 2010; 69: 801-816.
56. Louboutin JP, Reyes BA, Agrawal L, Van Bockstaele EJ, Strayer DS. HIV-1 gp120 upregulates matrix metalloproteinases and their inhibitors in a rat model of HIV encephalopathy. Eur J Neurosci 2011; 34: 2015-2023.
57. Eugenin EA, Clements JE, Zink MC, Berman JW. Human immunodeficiency virus infection of human astrocytes disrupts blood-brain barrier integrity by a gap junction-dependent mechanism. J Neurosci 2011; 31: 9456-9465.
58. Muratori C, Mangino G, Affabris E, Federico M. Astrocytes contacting HIV-1-infected macrophages increase the release of CCL2 in response to the HIV-1-dependent enhancement of membrane-associated TNFalpha in macrophages. Glia 2010; 58: 1893-1904.
59. Nakagawa S, Castro V, Toborek M. Infection of human pericytes by HIV-1 disrupts the integrity of the blood-brain barrier. J Cell Mol Med 2012; 16: 2950-2957.
60. Dutta R, Roy S. Mechanism(s) involved in opioid drug abuse modulation of HAND. Curr HIV Res 2012; 10: 469-477.
61. Ownby RL, Kumar AM, Benny Fernandez J, Moleon-Borodowsky I, Gonzalez L, Eisdorfer S, Waldrop-Valverde D, Kumar M. Tumor necrosis factor-alpha levels in HIV-1 seropositive injecting drug users. J Neuroimmune Pharmacol 2009; 4: 350-358.
62. Silverstein PS, Shah A, Weemhoff J, Kumar S, Singh DP, Kumar A. HIV-1 gp120 and drugs of abuse: interactions in the central nervous system. Curr HIV Res 2012; 10: 369-383.
63. Rogers TJ. Immunology as it pertains to drugs of abuse, AIDS and the neuroimmune axis: mediators and traffic. J Neuroimmune Pharmacol 2011; 6: 20-27.
64. Nath A. Human immunodeficiency virus-associated neurocognitive disorder: pathophysiology in relation to drug addiction. Ann N Y Acad Sci 2010; 1187: 122-128.
65. Yamamoto BK, Moszczynska A, Gudelsky GA. Amphetamine toxicities: classical and emerging mechanisms. Ann N Y Acad Sci 2010; 1187: 101-121.
66. Hollenbach R, Sagar D, Khan ZK, Callen S, Yao H, Shirazi J, Buch S, Jain P. Effect of morphine and SIV on dendritic cell trafficking into the central nervous system of rhesus macaques. J Neurovirol 2014; 20: 175-183.
67. Kousik SM, Napier TC, Carvey PM. The effects of psychostimulant drugs on blood brain barrier function and neuroinflammation. Front Pharmacol 2012; 3: 121.
68. Dietrich JB. Alteration of blood-brain barrier function by methamphetamine and cocaine. Cell Tissue Res 2009; 336: 385-392.
69. Cisneros IE, Ghorpade A. HIV-1, methamphetamine and astrocyte glutamate regulation: combined excitotoxic implications for neuro-AIDS. Curr HIV Res 2012; 10: 392-406.
70. Silverstein PS, Shah A, Gupte R, Liu X, Piepho RW, Kumar S, Kumar A. Methamphetamine toxicity and its implications during HIV-1 infection. J Neurovirol 2011; 17: 401-415.
71. Conant K, Lim ST, Randall B, Maguire-Zeiss KA. Matrix metalloproteinase dependent cleavage of cell adhesion molecules in the pathogenesis of CNS dysfunction with HIV and methamphetamine. Curr HIV Res 2012; 10: 384-391.
72. Buch S, Yao H, Guo M, Mori T, Mathias-Costa B, Singh V, Seth P, Wang J, Su TP. Cocaine and HIV-1 interplay in CNS: cellular and molecular mechanisms. Curr HIV Res 2012; 10: 425-428.
73. Yao H, Kim K, Duan M, Hayashi T, Guo M, Morgello S, Prat A, Wang J, Su TP, Buch S. Cocaine hijacks sigma1 receptor to initiate induction of activated leukocyte cell adhesion molecule: implication for increased monocyte adhesion and migration in the CNS. J Neurosci 2011; 31: 5942-5955.
74. Yao H, Duan M, Buch S. Cocaine-mediated induction of platelet-derived growth factor: implication for increased vascular permeability. Blood 2011; 117: 2538-2547.
75. Yao H, Bethel-Brown C, Niu F, Yang L, Peng F, Buch S. Yin and Yang of PDGF-mediated signaling pathway in the context of HIV infection and drug abuse. J Neuroimmune Pharmacol 2014; 9: 161-167.
76. Yao H, Duan M, Hu G, Buch S. Platelet-derived growth factor B chain is a novel target gene of cocaine-mediated Notch1 signaling: implications for HIV-associated neurological disorders. J Neurosci 2011; 31: 12449-12454.
77. Lu TS, Avraham HK, Seng S, Tachado SD, Koziel H, Makriyannis A, Avraham S. Cannabinoids inhibit HIV-1 Gp120-mediated insults in brain microvascular endothelial cells. J Immunol 2008; 181: 6406-6416.
78. Fraga D, Raborn ES, Ferreira GA, Cabral GA. Cannabinoids inhibit migration of microglial-like cells to the HIV protein Tat. J Neuroimmune Pharmacol 2011; 6: 566-577.
79. Gorantla S, Makarov E, Roy D, Finke-Dwyer J, Murrin LC, Gendelman HE, Poluektova L. Immunoregulation of a CB2 receptor agonist in a murine model of neuroAIDS. J Neuroimmune Pharmacol 2010; 5: 456-468.
80. Huang SH, Wang L, Chi F, Wu CH, Cao H, Zhang A, Jong A. Circulating brain microvascular endothelial cells (cBMECs) as potential biomarkers of the blood-brain barrier disorders caused by microbial and non-microbial factors. PLoS One 2013; 8: e62164.
81. Manda VK, Mittapalli RK, Bohn KA, Adkins CE, Lockman PR. Nicotine and cotinine increases the brain penetration of saquinavir in rat. J Neurochem 2010; 115: 1495-1507.
82. Manda VK, Mittapalli RK, Geldenhuys WJ, Lockman PR. Chronic exposure to nicotine and saquinavir decreases endothelial Notch-4 expression and disrupts blood-brain barrier integrity. J Neurochem 2010; 115: 515-525.
83. Persidsky Y, Ho W, Ramirez SH, Potula R, Abood ME, Unterwald E, Tuma R. HIV-1 infection and alcohol abuse: neurocognitive impairment, mechanisms of neurodegeneration and therapeutic interventions. Brain Behav Immun 2011; 25 Suppl 1: S61-70.

84. Marcondes MC, Flynn C, Huitron-Rezendiz S, Watry DD, Zandonatti M, Fox HS. Early antiretroviral treatment prevents the development of central nervous system abnormalities in simian immunodeficiency virus-infected rhesus monkeys. AIDS 2009; 23: 1187-1195.
85. Annamalai L, Bhaskar V, Pauley DR, Knight H, Williams K, Lentz M, Ratai E, Westmoreland SV, Gonzalez RG, O'Neil SP. Impact of short-term combined antiretroviral therapy on brain virus burden in simian immunodeficiency virus-infected and CD8+ lymphocyte-depleted rhesus macaques. Am J Pathol 2010; 177: 777-791.
86. Wilson MJ, Martin-Engel L, Vassileva J, Gonzalez R, Martin EM. An investigation of the effects of antiretroviral central nervous system penetration effectiveness on procedural learning in HIV+ drug users. J Clin Exp Neuropsychol 2013; 35: 915-925.
87. Webb KM, Mactutus CF, Booze RM. The ART of HIV therapies: dopaminergic deficits and future treatments for HIV pediatric encephalopathy. Expert Rev Anti Infect Ther 2009; 7: 193-203.
88. Eden A, Fuchs D, Hagberg L, Nilsson S, Spudich S, Svennerholm B, Price RW, Gisslen M. HIV-1 viral escape in cerebrospinal fluid of subjects on suppressive antiretroviral treatment. J Infect Dis 2010; 202: 1819-1825.
89. Peluso MJ, Ferretti F, Peterson J, Lee E, Fuchs D, Boschini A, Gisslen M, Angoff N, Price RW, Cinque P, Spudich S. Cerebrospinal fluid HIV escape associated with progressive neurologic dysfunction in patients on antiretroviral therapy with well controlled plasma viral load. AIDS 2012; 26: 1765-1774.
90. Eisfeld C, Reichelt D, Evers S, Husstedt I. CSF penetration by antiretroviral drugs. CNS Drugs 2013; 27: 31-55.
91. Calcagno A, Cusato J, Simiele M, Motta I, Audagnotto S, Bracchi M, D'Avolio A, Di Perri G, Bonora S. High interpatient variability of raltegravir CSF concentrations in HIV-positive patients: a pharmacogenetic analysis. J Antimicrob Chemother 2014; 69: 241-245.
92. Best BM, Letendre SL, Brigid E, Clifford DB, Collier AC, Gelman BB, McArthur JC, McCutchan JA, Simpson DM, Ellis R, Capparelli EV, Grant I. Low atazanavir concentrations in cerebrospinal fluid. AIDS 2009; 23: 83-87.
93. Rao KS, Ghorpade A, Labhasetwar V. Targeting anti-HIV drugs to the CNS. Expert Opin Drug Deliv 2009; 6: 771-784.
94. Ellis RJ, Letendre S, Vaida F, Haubrich R, Heaton RK, Sacktor N, Clifford DB, Best BM, May S, Umlauf A, Cherner M, Sanders C, Ballard C, Simpson DM, Jay C, McCutchan JA. Randomized trial of central nervous system-targeted antiretrovirals for HIV-associated neurocognitive disorder. Clin Infect Dis 2014; 58: 1015-1022.
95. Romao PR, Lemos JC, Moreira J, de Chaves G, Moretti M, Castro AA, Andrade VM, Boeck CR, Quevedo J, Gavioli EC. Anti-HIV drugs nevirapine and efavirenz affect anxiety-related behavior and cognitive performance in mice. Neurotox Res 2011; 19: 73-80.
96. Manda KR, Banerjee A, Banks WA, Ercal N. Highly active antiretroviral therapy drug combination induces oxidative stress and mitochondrial dysfunction in immortalized human blood-brain barrier endothelial cells. Free Radic Biol Med 2011; 50: 801-810.
97. Ashraf T, Kis O, Banerjee N, Bendayan R. Drug transporters at brain barriers: expression and regulation by neurological disorders. Adv Exp Med Biol 2012; 763: 20-69.
98. Al-Ghananeem AM, Smith M, Coronel ML, Tran H. Advances in brain targeting and drug delivery of anti-HIV therapeutic agents. Expert Opin Drug Deliv 2013; 10: 973-985.
99. Zhong Y, Hennig B, Toborek M. Intact lipid rafts regulate HIV-1 Tat protein-induced activation of the Rho signaling and upregulation of P-glycoprotein in brain endothelial cells. J Cereb Blood Flow Metab 2010; 30: 522-533.
100. Ashraf T, Robillard K, Chan G, Bendayan R. Role of CNS transporters in the pharmacotherapy of HIV-1 associated neurological disorders. Curr Pharm Des 2014; 20: 1543-1563.
101. Varatharajan L, Thomas SA. The transport of anti-HIV drugs across blood-CNS interfaces: summary of current knowledge and recommendations for further research. Antiviral Res 2009; 82: A99-109.
102. Sarginson JE, Lazzeroni LC, Ryan HS, Ershoff BD, Schatzberg AF, Murphy GM, Jr. ABCB1 (MDR1) polymorphisms and antidepressant response in geriatric depression. Pharmacogenet Genomics 2010; 20: 467-475.
103. Johnson DH, Sutherland D, Acosta EP, Erdem H, Richardson D, Haas DW. Genetic and non-genetic determinants of raltegravir penetration into cerebrospinal fluid: a single arm pharmacokinetic study. PLoS One 2013; 8: e82672.
104. Chan GN, Patel R, Cummins CL, Bendayan R. Induction of p-glycoprotein by antiretroviral drugs in human brain microvessel endothelial cells. Antimicrob Agents Chemother 2013; 57: 4481-4488.
105. Zastre JA, Chan GN, Ronaldson PT, Ramaswamy M, Couraud PO, Romero IA, Weksler B, Bendayan M, Bendayan R. Up-regulation of P-glycoprotein by HIV protease inhibitors in a human brain microvessel endothelial cell line. J Neurosci Res 2009; 87: 1023-1036.
106. Robillard KR, Chan GN, Zhang G, la Porte C, Cameron W, Bendayan R. Role of P-glycoprotein in the distribution of the HIV-protease inhibitor, atazanavir, in the brain and male genital tract. Antimicrob Agents Chemother 2014; 58: 1713-1722.
107. Roy U, Bulot C, Honer zu Bentrup K, Mondal D. Specific increase in MDR1 mediated drug-efflux in human brain endothelial cells following co-exposure to HIV-1 and saquinavir. PLoS One 2013; 8: e75374.
108. Calcagno A, Yilmaz A, Cusato J, Simiele M, Bertucci R, Siccardi M, Marinaro L, D'Avolio A, Di Perri G, Bonora S. Determinants of darunavir cerebrospinal fluid concentrations: impact of once-daily dosing and pharmacogenetics. AIDS 2012; 26: 1529-1533.

109. Dallas S, Block ML, Thompson DM, Bonini MG, Ronaldson PT, Bendayan R, Miller DS. Microglial activation decreases retention of the protease inhibitor saquinavir: implications for HIV treatment. J Neuroinflammation 2013; 10: 58.
110. Ene L, Duiculescu D, Ruta SM. How much do antiretroviral drugs penetrate into the central nervous system? J Med Life 2011; 4: 432-439.
111. Marzolini C, Mueller R, Li-Blatter X, Battegay M, Seelig A. The brain entry of HIV-1 protease inhibitors is facilitated when used in combination. Mol Pharm 2013; 10: 2340-2349.
112. Sagar V, Pilakka-Kanthikeel S, Pottathil R, Saxena SK, Nair M. Towards nanomedicines for neuroAIDS. Rev Med Virol 2014; 24: 103-124.
113. Wong HL, Wu XY, Bendayan R. Nanotechnological advances for the delivery of CNS therapeutics. Adv Drug Deliv Rev 2012; 64: 686-700.
114. Seremeta KP, Chiappetta DA, Sosnik A. Poly(epsilon-caprolactone), Eudragit(R) RS 100 and poly(epsilon-caprolactone)/Eudragit(R) RS 100 blend submicron particles for the sustained release of the antiretroviral efavirenz. Colloids Surf B Biointerfaces 2013; 102: 441-449.
115. Pathan SA, Iqbal Z, Zaidi SM, Talegaonkar S, Vohra D, Jain GK, Azeem A, Jain N, Lalani JR, Khar RK, Ahmad FJ. CNS drug delivery systems: novel approaches. Recent Pat Drug Deliv Formul 2009; 3: 71-89.
116. Namanja HA, Emmert D, Hrycyna CA, Chmielewski J. Homodimers of the antiviral abacavir as modulators of P-glycoprotein transport in cell culture: probing tether length. Medchemcomm 2013; 4: doi: 10.1039/C3MD00196B.
117. Saxena SK, Tiwari S, Nair MP. Nanotherapeutics: emerging competent technology in neuroAIDS and CNS drug delivery. Nanomedicine (Lond) 2012; 7: 941-944.
118. Namanja HA, Emmert D, Davis DA, Campos C, Miller DS, Hrycyna CA, Chmielewski J. Toward eradicating HIV reservoirs in the brain: inhibiting P-glycoprotein at the blood-brain barrier with prodrug abacavir dimers. J Am Chem Soc 2012; 134: 2976-2980.
119. Wong HL, Chattopadhyay N, Wu XY, Bendayan R. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. Adv Drug Deliv Rev 2010; 62: 503-517.
120. Ragnaill MN, Brown M, Ye D, Bramini M, Callanan S, Lynch I, Dawson KA. Internal benchmarking of a human blood-brain barrier cell model for screening of nanoparticle uptake and transcytosis. Eur J Pharm Biopharm 2011; 77: 360-367.
121. Syed S, Zubair A, Frieri M. Immune response to nanomaterials: implications for medicine and literature review. Curr Allergy Asthma Rep 2013; 13: 50-57.
122. Nowacek A, Gendelman HE. NanoART, neuroAIDS and CNS drug delivery. Nanomedicine (Lond) 2009; 4: 557-574.
123. Zensi A, Begley D, Pontikis C, Legros C, Mihoreanu L, Wagner S, Buchel C, von Briesen H, Kreuter J. Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones. J Control Release 2009; 137: 78-86.
124. Mahajan SD, Roy I, Xu G, Yong KT, Ding H, Aalinkeel R, Reynolds J, Sykes D, Nair BB, Lin EY, Prasad PN, Schwartz SA. Enhancing the delivery of anti retroviral drug "Saquinavir" across the blood brain barrier using nanoparticles. Curr HIV Res 2010; 8: 396-404.
125. Mahajan SD, Law WC, Aalinkeel R, Reynolds J, Nair BB, Yong KT, Roy I, Prasad PN, Schwartz SA. Nanoparticle-mediated targeted delivery of antiretrovirals to the brain. Methods Enzymol 2012; 509: 41-60.
126. Kanmogne GD, Singh S, Roy U, Liu X, McMillan J, Gorantla S, Balkundi S, Smith N, Alnouti Y, Gautam N, Zhou Y, Poluektova L, Kabanov A, Bronich T, Gendelman HE. Mononuclear phagocyte intercellular crosstalk facilitates transmission of cell-targeted nanoformulated antiretroviral drugs to human brain endothelial cells. Int J Nanomedicine 2012; 7: 2373-2388.
127. Bressani RF, Nowacek AS, Singh S, Balkundi S, Rabinow B, McMillan J, Gendelman HE, Kanmogne GD. Pharmacotoxicology of monocyte-macrophage nanoformulated antiretroviral drug uptake and carriage. Nanotoxicology 2011; 5: 592-605.
128. Dou H, Grotepas CB, McMillan JM, Destache CJ, Chaubal M, Werling J, Kipp J, Rabinow B, Gendelman HE. Macrophage delivery of nanoformulated antiretroviral drug to the brain in a murine model of neuroAIDS. J Immunol 2009; 183: 661-669.
129. Vinogradov SV, Poluektova LY, Makarov E, Gerson T, Senanayake MT. Nano-NRTIs: efficient inhibitors of HIV type-1 in macrophages with a reduced mitochondrial toxicity. Antivir Chem Chemother 2010; 21: 1-14.
130. Kuo YC, Chung CY. Transcytosis of CRM197-grafted polybutylcyanoacrylate nanoparticles for delivering zidovudine across human brain-microvascular endothelial cells. Colloids Surf B Biointerfaces 2012; 91: 242-249.
131. Saiyed ZM, Gandhi NH, Nair MP. AZT 5'-triphosphate nanoformulation suppresses human immunodeficiency virus type 1 replication in peripheral blood mononuclear cells. J Neurovirol 2009; 15: 343-347.
132. Nair M, Guduru R, Liang P, Hong J, Sagar V, Khizroev S. Externally controlled on-demand release of anti-HIV drug using magneto-electric nanoparticles as carriers. Nat Commun 2013; 4: 1707.
133. Kuo YC, Lu CH. Modulation of efflux proteins by electromagnetic field for delivering azidothymidine and saquinavir into the brain. Colloids Surf B Biointerfaces 2012; 91: 291-295.
134. Saiyed ZM, Gandhi NH, Nair MP. Magnetic nanoformulation of azidothymidine 5'-triphosphate for targeted delivery across the blood-brain barrier. Int J Nanomedicine 2010; 5: 157-166.
135. Im J, Kim W, Kim KT, Chung SK. Preparation of a 3'-azido-3'-deoxythymidine (AZT) derivative, which is blood-brain barrier permeable. Chem Commun (Camb) 2009; (31): 4669-4671.
136. Kuo YC, Lee CL. Methylmethacrylate-sulfopropylmethacrylate nanoparticles with surface RMP-7 for targeting delivery of antiretroviral drugs across the blood-brain barrier. Colloids Surf B Biointerfaces 2012; 90: 75-82.

137. Pilakka-Kanthikeel S, Atluri VS, Sagar V, Saxena SK, Nair M. Targeted brain derived neurotropic factors (BDNF) delivery across the blood-brain barrier for neuro-protection using magnetic nano carriers: an in-vitro study. PLoS One 2013; 8: e62241.
138. Lin CM, Lu TY. C60 fullerene derivatized nanoparticles and their application to therapeutics. Recent Pat Nanotechnol 2012; 6: 105-113.
139. Salcedo Gomez PM, Amano M, Yashchuk S, Mizuno A, Das D, Ghosh AK, Mitsuya H. GRL-04810 and GRL-05010, difluoride-containing nonpeptidic HIV-1 protease inhibitors (PIs) that inhibit the replication of multi-PI-resistant HIV-1 in vitro and possess favorable lipophilicity that may allow blood-brain barrier penetration. Antimicrob Agents Chemother 2013; 57: 6110-6121.
140. Nagle MA, Wu W, Eraly SA, Nigam SK. Organic anion transport pathways in antiviral handling in choroid plexus in Oat1 (Slc22a6) and Oat3 (Slc22a8) deficient tissue. Neurosci Lett 2013; 534: 133-138.
141. Mehla R, Bivalkar-Mehla S, Chauhan A. A flavonoid, luteolin, cripples HIV-1 by abrogation of tat function. PLoS One 2011; 6: e27915.
142. Manak MM, Moshkoff DA, Nguyen LT, Meshki J, Tebas P, Tuluc F, Douglas SD. Anti-HIV-1 activity of the neurokinin-1 receptor antagonist aprepitant and synergistic interactions with other antiretrovirals. AIDS 2010; 24: 2789-2796.
143. Mahajan HS, Mahajan MS, Nerkar PP, Agrawal A. Nanoemulsion-based intranasal drug delivery system of saquinavir mesylate for brain targeting. Drug Deliv 2014; 21: 148-154.
144. Clark US, Cohen RA. Brain dysfunction in the era of combination antiretroviral therapy: implications for the treatment of the aging population of HIV-infected individuals. Curr Opin Investig Drugs 2010; 11: 884-900.
145. Andras IE, Toborek M. Amyloid beta accumulation in HIV-1-infected brain: The role of the blood brain barrier. IUBMB Life 2013; 65: 43-49.
146. Andras IE, Eum SY, Huang W, Zhong Y, Hennig B, Toborek M. HIV-1-induced amyloid beta accumulation in brain endothelial cells is attenuated by simvastatin. Mol Cell Neurosci 2010; 43: 232-243.