HIV and viral protein effects on the blood brain barrier

MaryPeace McRae*

Department of Pharmacotherapy and Outcomes Sciences; Virginia Commonwealth University; Richmond, VA USA

Keywords: blood brain barrier, HIV, P-glycoprotein, tight junctions, transporters

Abbreviations and Acronyms: BBB, Blood brain barrier; CNS, Central Nervous System; CSF, Cerebral Spinal Fluid; HIV, Human Immunodeficiency Virus; cART, Combination Antiretroviral Therapy; ZO-1, Zonula Occludens-1; JAM-2, Junctional Adhesion Molecule; MRP, Multidrug Resistance Protein

The blood brain barrier (BBB) plays a critical role in the normal physiology of the central nervous system (CNS) by regulating what crosses from the periphery into the brain. Damage to the BBB or alterations in transport systems may mediate the pathogenesis of many CNS diseases, including HIV-associated CNS dysfunction. HIV-1 infection can result in neuropathologic changes in about one half of infected individuals and also can result in damage to the BBB. HIV-1 and the HIV-1 viral proteins, Tat and gp120, cause alterations in the integrity and function of the BBB through both paracellular and transcellular mechanisms. The current review discusses HIV and viral protein-mediated injury to the BBB with a focus on the effects on tight junction proteins, barrier permeability, and drug efflux proteins.

Introduction

Despite aggressive use of combination antiretroviral therapy (cART), HIV-1 infection results in HIV encephalitis and neurobehavioral impairment, collectively termed neuroAIDS, in about half of infected individuals.¹⁻⁶ Neurocognitive dysfunction includes decreased attention and concentration, information processing, memory, learning and psychomotor speed. There may also be motor slowing, incoordination and tremor.^{1,7} The clinical severity of HIV-associated neurocognitive disorders (HAND) can range from asymptomatic neurocognitive impairment to full blown HIV-associated dementia. Although the severity of neuroAIDS syndromes have diminished in the post-cART era, the incidence of HIV-associated neurocognitive disorders and debilitating deficits in neurobehavioral function persist as HIV/AIDS evolves into a chronic disease.^{5,8}

One consequence of this chronic disease process is that HIV-1 and HIV-1 viral proteins cause inflammatory responses (increase in inflammatory cytokines and chemokines) in many cell types in the periphery and within the

central nervous system (CNS).⁹⁻¹¹ HIV-1, viral proteins, and the inflammatory mediators result in structural and functional damage within the CNS and to the blood brain barrier (BBB). The resultant dysfunction of the BBB can allow, through multiple mechanisms, for increased passage of HIV-1 infected cells into the brain, can alter the barrier's permeability to small water-soluble compounds and may also alter the penetration of therapeutic drugs into the brain.¹²⁻¹⁵

Blood brain barrier

The BBB is a selective barrier composed of microvascular endothelial cells lining the brain microvessels. The BBB has an active interface between the circulation and the CNS, restricts free movement of substances between blood and CNS, and is critical in maintaining CNS homeostasis.¹⁶ The endothelial cells are surrounded by basal lamina, pericytes and the endfeet of perivascular astrocytes. Pericytes and astrocytes are involved in the regulation of the BBB. Pericytes regulate BBB permeability, tight junction expression, and vesicle trafficking within the endothelial cells^{17,18} and the lack of pericytes results in increased barrier permeability.^{19,20} Astrocytes also contribute to the induction and maintenance of BBB properties through modulation of tight junction expression, enzyme induction, and polarized localization of transport proteins, such as P-glycoprotein and GLUT1.²¹⁻²⁶

The barrier function is maintained by several mechanisms. A paracellular barrier is formed by tight junctions between endothelial cells that restrict movement of water-soluble compounds between adjacent cells. A transcellular barrier is formed due to the low level of endocytosis and transcytosis of brain endothelial cells and serves to inhibit transport of substances through the cytoplasm. Furthermore, the BBB serves as an enzymatic barrier (drug metabolizing enzymes, acetylcholinesterase, alkaline phosphatase, gamma-glutamyl transpeptidase, monoamine oxidases) capable of degrading many chemical compounds. Lastly, endothelial cells express various efflux transporters (P-glycoprotein, Breast Cancer Resistance Protein or BCRP, some members of the Multidrug Resistance Protein (MRP) family) that are responsible for expulsion of substances from brain endothelial cell cytoplasm back into the blood, thereby limiting CNS exposure (Fig. 1).¹⁶

*Correspondence to: MaryPeace McRae; Email: mpmcrae@vcu.edu
Submitted: 11/03/2015; Revised: 01/09/2016; Accepted: 01/12/2016
<http://dx.doi.org/10.1080/21688370.2016.1143543>

Figure 1. HIV and the blood brain barrier. A representation of the routes of transport across the BBB, including routes by which HIV and viral proteins traverse the barrier. Tight junctions limit the penetration of water-soluble compounds (6). However, lipid-soluble compounds can diffuse across the endothelial lipid membrane (5). The endothelium contains transport proteins including active efflux proteins such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) that limit penetration across the barrier and extrude compounds back into blood (3). Some proteins are able to cross the barrier by receptor mediated endocytosis and transcytosis (7). HIV-1 viral proteins, tat and gp120 traverse the barrier via adsorptive endocytosis (1). Much of the HIV-1 that traverses the BBB does so inside infected monocytes (2) but free virus also can penetrate the barrier via openings in tight junctions (4) or transcytosis (8). Viral proteins and HIV infection cause the release of cytokines, chemokines and additional viral proteins that can result in damage to the BBB. Adapted from.^{21,84,99}

The brain microvascular endothelial cells form continuous capillaries and lack fenestra or sinusoids typical of most capillaries elsewhere. Continuous complexes of tight junctions form a belt-like structure between cells that interconnect endothelial cells. The continuous tight junctional complex provides a physical barrier forcing most molecular traffic to take a transcellular route across the BBB, which can be actively regulated by transporters, rather than paracellularly through the gaps in the junctional complex as in non-continuous capillaries.^{16,21} The BBB permits maintenance of a unique extracellular microenvironment that is essential for normal CNS function.

HIV, HIV viral proteins and the BBB

HIV infection results in alterations in the BBB integrity, tight junction expression, and also results in increases in transmigration of cells across the barrier. Early studies on the effects of HIV-1 within the brain were performed on post-mortem brain samples from patients with AIDS. These studies revealed elevations in markers for vascular permeability and morphologic abnormalities consistent with dysfunction of the BBB.²⁷⁻²⁹ Cytokines and chemokines are elevated in the cerebral spinal fluid (CSF) of patients with HIV-associated dementia as compared to HIV-infected individuals without dementia. Additionally, brain tissue from HIV-infected patients has higher expression of adhesion molecules, such as platelet endothelial cell adhesion molecule (PECAM).³⁰

In vitro data support and extend the findings from patient samples. HIV-1 infection increases adhesion molecule expression,³⁰⁻³² increases monocyte transmigration across cultured

endothelial cells,³³⁻⁴⁰ and alters cytokine expression and interferon signaling in human brain endothelial cells.⁴¹

These alterations, as well as other mechanisms, may contribute to the perturbations in BBB tight junction expression observed in response to HIV. Exposure to HIV results in decreases in ZO-1, claudin-5 and occludin expression.^{34,41-45} These changes are mediated in part, by *STAT1* signaling through JAK/STAT pathway and Rho kinase phosphorylation of tight junctions.^{34,41,44,45} Inhibition of STAT by the inhibitor fludarabine blocks HIV-1 induced changes in tight junctions and blocks HIV-1 induced increases in monocyte migration.⁴⁵ Brain autopsies from patients with HIV-1 associated dementia reveals diminished expression of claudin-5 and enhanced expression of *STAT1*.⁴⁵ HIV infection results in perturbations in numerous normal brain processes. Many of these alterations can, either directly or indirectly, cause damage to the BBB and may result in

increased leakiness of the barrier.

HIV-1 Tat

Tat (trans-activator of transcription) is a viral protein produced by HIV-infected cells that enhances HIV gene transcription and replication.^{46,47} Tat is released from HIV-1 infected cells and can be detected in the CNS and the serum of HIV-infected patients.⁴⁸⁻⁵¹ Tat is a promiscuous ligand, its receptors are ubiquitous, and therefore Tat can enter several cell types. It does so via endocytic internalization.⁵² Once inside cells, Tat activates a variety of cellular genes. Within brain endothelial cells, Tat increases the expression of chemokine receptors, induces cytokine release, induces oxidative stress, results in monocyte chemoattractant protein-1 (MCP-1) release and promotes immune cell permeation across the BBB.^{12,14,15}

In *in vitro* and *in vivo* models, Tat has been shown to alter the expression of BBB tight junction proteins, with decreases in ZO-1, ZO-2, occludin and claudin-5^{13,53-58} and increases in JAM-2 expression.^{13,55} Exposure to Tat also increases barrier permeability to paracellular compounds such as Evans Blue or FITC-dextran⁵³⁻⁶⁰ and results in increases in the transmigration of monocytes across the barrier.^{13,14,38,61,64}

Tat effects on tight junction protein expression and barrier function are mediated through multiple signaling pathways. Studies have found that Tat can interact with G-protein coupled receptors (GPCRs) such as vascular endothelial growth factor receptor-2 (VEGFR-2), leading to decreases in claudin-5 expression.⁵⁷ Activation of VEGFR-2 leads to downstream activation of other signaling molecules, such as MEK1/2. Additionally Tat

alters claudin-5 via activation of PI-3K and NF- κ B.⁵⁷ Ras, caveolin-1, COX-2, Rho signaling and activation of transcription factor cAMP response element-binding protein (CREB) are all pathways that have been implicated in Tat-mediated effects on ZO-1, ZO-2 or occludin expression.^{53,54,65}

gp120

gp120 is an envelope protein required for viral entry. It facilitates viral replication and disease progression.⁶⁶ It is a potent neurotoxin and has been detected in the serum of HIV-infected patients.⁶⁷ Like Tat, gp120 crosses the BBB via adsorptive endocytosis (Fig. 1).⁶⁸ Within the brain, gp120 is directly neurotoxic to neurons, but also has indirect effects via increases in inflammatory cytokine production, increases in oxidative stress, and increased BBB permeability.^{69,70}

The increases in BBB permeability with gp120 exposure is also associated with alterations in tight junction expression, increased stress fiber formation and morphological changes in brain microvascular endothelial cells.⁷¹⁻⁷⁶

Rat brain endothelial cells exposed to gp120 for 24 hours results in increased permeability to albumin. This effect can be blocked by co-incubation with gp120 antibody and by the substance P inhibitor, spantide.⁷⁷ gp120-associated increases in albumin permeability has also been demonstrated in normal mouse brain endothelial cell cultures and in gp120 transgenic mice.^{78,79} In addition, gp120 transgenic mice have increased vessel expression of the adhesion proteins PECAM and intercellular adhesion molecule (ICAM), which likely contribute to the enhanced migration of monocytes across the barrier.⁷⁹

Human brain microvascular endothelial cells respond to gp120 in a similar way as rodent brain endothelial cells. It has been demonstrated that exposure to gp120 (derived from both CCR5 and CXCR4 tropic viruses) results in damage to the BBB, which can be restored after removal of gp120. Furthermore, these authors discovered that the gp120-induced stimulation of intracellular calcium release activated protein kinase C (PKC) pathways, and resulted in increased permeability of the barrier and enhanced monocyte migration.^{80,81} gp120-induced disruptions of the BBB also can occur via oxidative stress, activation of the NMDA receptor, activation of matrix metalloproteinases, and degradation of basement membrane.^{71,76}

HIV, HIV viral proteins, and drug efflux transporters

The major efflux transporters at the human BBB are P-glycoprotein (ABCB1), the breast cancer resistance protein (BCRP, ABCG2) and the multidrug resistance protein family (MRP, ABCC family). These efflux transporters are located on the apical membrane of the BBB and are responsible for the expulsion of substances from the brain back into the blood (Fig. 1).^{82,83} Many antiretroviral drugs used in the treatment of HIV are substrates, inducers and/or inhibitors of these transport proteins.⁸⁴⁻⁸⁶ Inhibition of these transporters, either pharmacologically or via genetic knockout mouse models, results in significant accumulation of substrates within the brain.⁸⁷⁻⁸⁹

HIV-1 and HIV viral proteins can modulate transporter expression and function at the BBB. Viral proteins and HIV

infection result in increases in cytokine, chemokine, and oxidative stress signals that can affect drug efflux transport protein expression and function in a variety of brain cells.^{9,10,70,75,76,90}

In post-mortem brain samples from HIV-infected patients, P-glycoprotein expression has been shown to be decreased in brain endothelial cells but increased in astrocytes and microglia.⁸⁶ However, these samples came from individuals taking antiretroviral therapy and therefore it could not be determined if the alterations in the transport proteins observed were a result of the infection or a result of the therapy.

In response to exposure to viral proteins, animal and *in vitro* data demonstrate increases in P-glycoprotein expression. In rat brain microvascular endothelial cells, Tat treatment caused increases in P-glycoprotein mRNA and protein expression as well as increases in cellular uptake of the P-glycoprotein substrate, Rhodamine 123.⁹² Astrocyte expression of P-glycoprotein was also increased by Tat. These findings were confirmed *in vivo* in brain vessels of mice injected with Tat.⁹² Exposure of human brain microvascular endothelial cells to HIV-1 Tat also results in increases in the mRNA expression of P-glycoprotein.¹³

As suggested above, the alterations in P-glycoprotein expression in response to HIV or viral proteins often, but not in all studies, appear to be cell-type specific. *In vitro*, mouse and human brain microvascular endothelial cells have increases in P-glycoprotein expression after exposure to HIV or Tat, but in primary human astrocytes, HIV and gp120 down-regulate P-glycoprotein expression and activity.^{93,94} Human post-mortem samples also demonstrate cell type specificity, with brain endothelial cells and astrocytes having opposite directionality in their responses.⁹¹

Fewer studies investigating the effects of HIV or viral proteins on members of the multidrug resistance protein (MRP) family have been conducted. One study investigated the effects of Tat exposure on MRP1-MRP7 expression in primary rat brain microvascular endothelial cells and primary mouse astrocytes. Treatment with Tat resulted in a significant increase in MRP1 mRNA and protein expression as well as increases in MRP1 function in endothelial cells and astrocytes. There were no statistically significant mRNA changes detected in the other MRP proteins (MRP2-MRP7) examined.⁹⁵ Another group demonstrated an increase in Mrp1, but not Mrp3 expression in primary rat astrocytes.⁷⁰

The regulatory pathways of drug transporters at the BBB are complex. It appears that HIV, and viral proteins can affect the regulation of efflux transporters via multiple mechanisms, including enhanced cytokine secretion, lipid rafts, RhoA/ROCK signaling, NF- κ B, and myosin light chain kinase, among others.^{92,93,96,97}

Conflicting results between studies attest to the complexity of HIV-BBB interactions. Variable outcomes of studies examining the effects of HIV and viral proteins on transporter expression within the cells of the BBB may be due to species differences, differences in doses or treatment durations and to the inherent differences between the models used. Although a critical tool for scientists, *in vitro* models are limited in their ability to mimic or capture all of the complexities of the factors contributing to HIV

associated human neurocognitive dysfunction. The nature of the critical intercellular relationships between the endothelial cells, astrocyte endfeet and pericytes may be distorted *in vitro*. Current BBB *in vitro* models range in complexity from static single cell models to more complex, flow dependent, 3-dimensional co-culture models.⁹⁸ It is tempting to try to assign one model as the 'best' model for the BBB. However, if the strengths and limitations of the model are kept in mind when designing and interpreting studies, many different models can be used effectively to investigate the various effects of HIV on the BBB.

Drug efflux transporters can be important determinants of drug distribution into the brain. Additionally, many antiretroviral drugs used in the treatment of HIV are substrates as well as inducers and/or inhibitors for these transporters. As detailed above, HIV and viral proteins can affect the expression and function of several important efflux proteins. However, further work in this area to examine the complex interactions of HIV infection and antiretroviral therapy should be performed. This work should concentrate not only on drug efflux proteins, but also on BBB structure, function and integrity, and with an emphasis on how these interactions affect antiretroviral *penetration* into the brain.

Conclusions/future perspectives

Our understanding of the effects of HIV-1 and virally secreted proteins on the integrity and function of the BBB is somewhat limited. It is clear that breakdown of the BBB occurs in response to HIV or the viral proteins Tat and gp120. This disruption results in alterations in tight junction protein expression, leading to enhanced flux of paracellular compounds across the barrier. Exposure to HIV and viral proteins also results in enhanced monocyte migration across the barrier and causes alterations in the expression and function of active efflux transport proteins such as P-glycoprotein. The mechanisms of how the various perturbations in the BBB occur are even less well known, but are likely quite complex. Furthermore, how the alterations in integrity and function of the BBB impact the delivery of antiretroviral drugs to the brain is an area of great import. Because the brain is a reservoir for HIV and delivery of antiretroviral drugs to the brain is crucial for adequate viral control, a better understanding of the disease-specific factors affecting antiretroviral penetration into the brain will help in the design of better CNS penetrating drug therapy and could lead to the elimination of HIV-associated neurologic disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

- Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Clifford DB, Cinque P, Epstein LG, Goodkin K, et al. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology* 2007; 69:1789-99; PMID:17914061; <http://dx.doi.org/10.1212/01.WNL.0000287431.88658.8b>
- McArthur JC, Steiner J, Sacktor N, Nath A. HIV-associated neurocognitive disorders: "mind the gap." *Ann Neurol* 2010; 67:699-714; PMID:20517932
- Sheppard DP, Iudicello JE, Bondi MW, Doyle KL, Morgan EE, Massman PJ, Gilbert PE, Woods SP. Elevated rates of mild cognitive impairment in HIV disease. *J Neurovirol* 2015; 21(5):576-84; PMID:26139019
- Watkins CC, Treisman GJ. Cognitive impairment in patients with AIDS – prevalence and severity. *HIV/AIDS-Research Palliat Care* 2015; 7:35-47; PMID:25678819; <http://dx.doi.org/10.2147/HIV.S39665>
- Vivithanaporn P, Gill MJ, Power C. Impact of current antiretroviral therapies on neuroAIDS. *Expert Rev Anti Infect Ther* 2011; 9:371-4; PMID:21504390; <http://dx.doi.org/10.1586/eri.10.179>
- Heaton RK, Clifford DB, Franklin DR, Woods SP, Ake C, Vaida F, Ellis RJ, Letendre SL, Marcotte TD, Atkinson JH, et al. HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER Study. *Neurology* 2010; 75:2087-96; PMID:21135382; <http://dx.doi.org/10.1212/WNL.0b013e318200d727>
- Alfahad TB, Nath A. Update on HIV-Associated Neurocognitive Disorders. *Curr Neurol Neurosci Rep* 2013; 13:387, 1-8; PMID:23954972
- Ellis R, Langford D, Masliah E. HIV and antiretroviral therapy in the brain: neuronal injury and repair. *Nat Rev Neurosci* 2007; 8:33-44; PMID:17180161; <http://dx.doi.org/10.1038/nrn2040>
- El-Hage N, Gurwell JA, Singh IN, Knapp PE, Nath A, Hauser KF. Synergistic increases in intracellular Ca²⁺, and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia* 2005; 50:91-106; PMID:15630704; <http://dx.doi.org/10.1002/glia.20148>
- El-Hage N, Bruce-Keller AJ, Yakovleva T, Bazov I, Bakalkin G, Knapp PE, Hauser KF. Morphine exacerbates HIV-1 Tat-induced cytokine production in astrocytes through convergent effects on [Ca²⁺]_i and NF-κB trafficking and transcription. *PLoS One* 2008; 3:e4093; PMID:19116667; <http://dx.doi.org/10.1371/journal.pone.0004093>
- Nath A, Conant K, Chen P, Scott C, Major EO. Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. *J Biol Chem* 1999; 274:17098-102; PMID:10358063; <http://dx.doi.org/10.1074/jbc.274.24.17098>
- Toborek M, Lee YW, Pu H, Malecki A, Flora G, Garrido R, Hennig B, Bauer H-C, Nath A. HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. *J Neurochem* 2003; 84:169-79; PMID:12485413; <http://dx.doi.org/10.1046/j.1471-4159.2003.01543.x>
- Mahajan SD, Aalincel R, Sykes DE, Reynolds JL, Bindukumar B, Fernandez SF, Chawda R, Shanahan TC, Schwartz SA. Tight junction regulation by morphine and HIV-1 tat modulates blood-brain barrier permeability. *J Clin Immunol* 2008; 28:528-41; PMID:18574677; <http://dx.doi.org/10.1007/s10875-008-9208-1>
- Weiss JM, Nath A, Major EO, Berman JW. HIV-1 Tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood-brain barrier and up-regulates CCR5 expression on human monocytes. *J Immunol* 1999; 163:2953-9; PMID:10453044
- Banks WA, Robinson SM, Nath A. Permeability of the blood-brain barrier to HIV-1 Tat. *Exp Neurol* 2005; 193:218-27; PMID:15817280; <http://dx.doi.org/10.1016/j.expneurol.2004.11.019>
- Wilhelm I, Fazakas C, Krizbai IA. In Vitro models of the blood-brain barrier. *Acta Neurobiol Exp (Wars)* 2011; 71:113-28; PMID:21499332
- Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 2010; 468:562-6; PMID:20944625; <http://dx.doi.org/10.1038/nature09513>
- Nakagawa S, Deli MA, Nakao S, Honda M, Hayashi K, Nakaoka R, Kataoka Y, Niwa M. Pericytes from Brain Microvessels Strengthen the Barrier Integrity in Primary Cultures of Rat Brain Endothelial Cells. *Cell Mol Neurobiol* 2007; 27:687-94; PMID:17823866; <http://dx.doi.org/10.1007/s10571-007-9195-4>
- Armulik E, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, et al. Pericytes regulate the blood-brain barrier. *Nature* 2010; 468:557-61; PMID:20944627; <http://dx.doi.org/10.1038/nature09522>
- Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic B V. Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 2010; 68:409-27; PMID:21040844; <http://dx.doi.org/10.1016/j.neuron.2010.09.043>
- Abbott NJ, Rönnebeck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 2006; 7:41-53; PMID:16371949; <http://dx.doi.org/10.1038/nrn1824>
- Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis* 2010; 37:13-25; PMID:19664713; <http://dx.doi.org/10.1016/j.nbd.2009.07.030>
- Hayashi Y, Nomura M, Yamagishi S, Harada S, Yamashita J, Yamamoto H. Induction of various blood-brain barrier properties in non-neural endothelial cells by close apposition to co-cultured astrocytes. *Glia* 1997; 19:13-26; PMID:8989564; [http://dx.doi.org/10.1002/\(SICI\)1098-1136\(199701\)19:1%3c13::AID-GLIA2%3e3.0.CO;2-B](http://dx.doi.org/10.1002/(SICI)1098-1136(199701)19:1%3c13::AID-GLIA2%3e3.0.CO;2-B)

24. Dehouck MP, Méresse S, Delorme P, Fruchart JC, Cecchelli R. An easier, reproducible, and mass-production method to study the blood-brain barrier in vitro. *J Neurochem* 1990; 54:1798-801; PMID:2182777; <http://dx.doi.org/10.1111/j.1471-4159.1990.tb01236.x>
25. Winger RC, Koblinki JE, Kanda T, Ransohoff RM, Muller WA. Rapid remodeling of tight junctions during paracellular diapedesis in a human model of the blood-brain barrier. *J Immunol* 2014; 193:2427-37; PMID:25063869; <http://dx.doi.org/10.4049/jimmunol.1400700>
26. Janzer RC, Raff MC. Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 1987; 325:253-7; PMID:3543687; <http://dx.doi.org/10.1038/325253a0>
27. Petit CK, Cash KS. Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain. *Ann Neurol* 1992; 32:658-66; PMID:1449246; <http://dx.doi.org/10.1002/ana.410320509>
28. Power C, Kong P a, Crawford TO, Wesselingh S, Glass JD, McArthur JC, Trapp BD. Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann Neurol* 1993; 34:339-50; PMID:7689819; <http://dx.doi.org/10.1002/ana.410340307>
29. Smith T, DeGirolami U, Henin D, Bolgert F, Hauw J. Human Immunodeficiency virus (HIV) Leukoencephalopathy and the Microcirculation. *J Neuropathol Exp Neurol* 1990; 49:357-70; PMID:2362185; <http://dx.doi.org/10.1097/00005072-199007000-00001>
30. Eugenin EA, Gamss R, Buckner C, Buono D, Klein RS, Schoenbaum E, Calderon T, Berman JW. Shedding of PECAM-1 during HIV infection: a potential role for soluble PECAM-1 in the pathogenesis of NeuroAIDS. *J Leukoc Biol* 2006; 79:444-52; PMID:16507710; <http://dx.doi.org/10.1189/jlb.0405215>
31. Nottet HS, Persidsky Y, Sasseville VG, Nukuna A N, Bock P, Zhai QH, Sharer LR, McComb RD, Swindells S, Soderland C, et al. Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol* 1996; 156:1284-95; PMID:8558009
32. Ricardo-Dukelow M, Kadiu I, Rozek W, Schlautman J, Persidsky Y, Ciborowski P, Kannmogne GD, Gendelman HE. HIV-1 infected monocyte-derived macrophages affect the human brain microvascular endothelial cell proteome: New insights into blood-brain barrier dysfunction for HIV-1-associated dementia. *J Neuroimmunol* 2007; 185:37-46; PMID:17321604; <http://dx.doi.org/10.1016/j.jneuroim.2007.01.004>
33. Saukkonen JJ, Furfaro S, Mahoney KM, Strieter RM, Burdick M, Wright E a, Kornfeld H, Berman JS. In vitro transendothelial migration of blood T lymphocytes from HIV-infected individuals. *AIDS* 1997; 11:1595-601; PMID:9365764; <http://dx.doi.org/10.1097/00002030-199713000-00008>
34. 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; 31:9456-65; PMID:21715610; <http://dx.doi.org/10.1523/JNEUROSCI.1460-11.2011>
35. Eugenin EA. CCL2/Monocyte Chemoattractant Protein-1 Mediates Enhanced Transmigration of Human Immunodeficiency Virus (HIV)-Infected Leukocytes across the Blood-Brain Barrier: A Potential Mechanism of HIV-CNS Invasion and NeuroAIDS. *J Neurosci* 2006; 26:1098-106; PMID:16436595; <http://dx.doi.org/10.1523/JNEUROSCI.3863-05.2006>
36. Persidsky Y, Zheng J, Miller D, Gendelman HE. Mononuclear phagocytes mediate blood-brain barrier compromise and neuronal injury during HIV-1-associated dementia. *J Leukoc Biol* 2000; 68:413-22; PMID:10985259
37. Persidsky Y, Ghorpade A, Rasmussen J, Limoges J, Liu XJ, Stins M, Fiala M, Way D, Kim KS, Witte MH, et al. Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am J Pathol* 1999; 155:1599-611; PMID:10550317; [http://dx.doi.org/10.1016/S0002-9440\(10\)65476-4](http://dx.doi.org/10.1016/S0002-9440(10)65476-4)
38. Buckner CM, Calderon TM, Williams 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; 267:109-23; PMID:2192246; <http://dx.doi.org/10.1016/j.cellimm.2010.12.004>
39. Coley JS, Calderon TM, Gaskill PJ, Eugenin EA, Berman JW. Dopamine Increases CD14+CD16+ Monocyte Migration and Adhesion in the Context of Substance Abuse and HIV Neuropathogenesis. *PLoS One* 2015; 10:e0117450; PMID:25647501; <http://dx.doi.org/10.1371/journal.pone.0117450>
40. 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; 91:401-15; PMID:2227964; <http://dx.doi.org/10.1189/jlb.0811394>
41. Chaudhuri A, Duan F, Morsey B, Persidsky Y, Kannmogne GD. HIV-1 activates proinflammatory and interferon-inducible genes in human brain microvascular endothelial cells: putative mechanisms of blood-brain barrier dysfunction. *J Cereb Blood Flow & Metab* 2008; 28:697-711; PMID:17940540; <http://dx.doi.org/10.1038/sj.jcbfm.9600567>
42. Dallasta LM, Pisarov LA, Esplen JE, Werley J V, Moses A V, Nelson JA, Achim CL. Blood-Brain Barrier Tight Junction Disruption in Human Immunodeficiency Virus-1 Encephalitis. *Am J Pathol* 1999; 155:1915-27; PMID:10595922; [http://dx.doi.org/10.1016/S0002-9440\(10\)65511-3](http://dx.doi.org/10.1016/S0002-9440(10)65511-3)
43. Boven LA, Middel J, Verhoef J, De Groot CJA, Nottet HSLM. Monocyte infiltration is highly associated with loss of the tight junction protein zonula occludens in HIV-1-associated dementia. *Neuropathol Appl Neurobiol* 2000; 26:356-60; PMID:10931369; <http://dx.doi.org/10.1046/j.1365-2990.2000.00255.x>
44. Persidsky Y, Heilman D, Haorah J, Zelivyanskaya M, Persidsky R, Weber GA, Shimokawa H, Kaibuchi K, Ikezu T. Rho-mediated regulation of tight junctions during monocyte migration across the blood-brain barrier in HIV-1 encephalitis (HIVE). *Blood* 2006; 107:4770-80; PMID:16478881; <http://dx.doi.org/10.1182/blood-2005-11-4721>
45. Chaudhuri A, Yang B, Gendelman HE, Persidsky Y, Georgette D, Kannmogne GD. STAT1 signaling modulates HIV-1 – induced inflammatory responses and leukocyte transmigration across the blood-brain barrier. *Blood* 2008; 111:2062-72; PMID:18003888; <http://dx.doi.org/10.1182/blood-2007-05-091207>
46. Kelly GD, Ensoli B, Gunthel CJ, Offermann MK. Purified Tat induces inflammatory response genes in Kaposi's sarcoma cells. *Aids* 1998; 12:1753-61; PMID:9792375; <http://dx.doi.org/10.1097/00002030-199814000-00006>
47. Pu H, Tian J, Flora G, Woo Lee Y, Nath A, Hennig B, Toborek M. HIV-1 tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. *Mol Cell Neurosci* 2003; 24:224-37; PMID:14550782; [http://dx.doi.org/10.1016/S1044-7431\(03\)00171-4](http://dx.doi.org/10.1016/S1044-7431(03)00171-4)
48. Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debatin KM, Krammer PH. Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* 1995; 375:497-500; PMID:7539892; <http://dx.doi.org/10.1038/375497a0>
49. Xiao H, Neuveut C, Tiffany HL, Benkirane M, Rich EA, Murphy PM, Jeang KT. Selective CXCR4 antagonism by Tat: implications for in vivo expansion of coreceptor use by HIV-1. *Proc Natl Acad Sci United States Am* 2000; 97:11466-71; PMID:11027346; <http://dx.doi.org/10.1073/pnas.97.21.11466>
50. Hudson L, Liu J, Nath A, Jones M, Raghavan R, Narayan O, Male D, Everall I. Detection of the human immunodeficiency virus regulatory protein tat in CNS tissues. *J Neurovirol* 2000; 6:145-55; PMID:10822328; <http://dx.doi.org/10.3109/13550280009013158>
51. Wiley CA, Baldwin M, Achim CL. Expression of HIV regulatory and structural mRNA in the central nervous system. *AIDS* 1996; 10:843-7; PMID:8828741; <http://dx.doi.org/10.1097/00002030-199607000-00007>
52. Debaisieux S, Rayne F, Yezid H, Beaumelle B. The Ins and Outs of HIV-1 Tat. *Traffic* 2011; 13:355-63; PMID:21951552; <http://dx.doi.org/10.1111/j.1600-0854.2011.01286.x>
53. Pu H, Hayashi K, Andras IE, Eum S, Hennig B, Toborek M. Limited role of COX-2 in HIV Tat-induced alterations of tight junction protein expression and disruption of the blood-brain barrier. *Brain Res* 2007; 1184:333-44; PMID:17976544; <http://dx.doi.org/10.1016/j.brainres.2007.09.063>
54. Zhong Y, Smart EJ, Weksler B, Couraud P-O, Hennig B, Toborek M. Caveolin-1 regulates human immunodeficiency virus-1 Tat-induced alterations of tight junction protein expression via modulation of the Ras signaling. *J Neurosci* 2008; 28:7788-96; PMID:18667611; <http://dx.doi.org/10.1523/JNEUROSCI.0061-08.2008>
55. Gandhi N, Saiyed ZM, Napuri J, Samikkannu T, Reddy PVB, Agudelo M, Khatavkar P, Saxena SK, Nair MPN. 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; PMID:20624003; <http://dx.doi.org/10.3109/13550284.2010.499891>
56. 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; 1436:13-9; PMID:22197032; <http://dx.doi.org/10.1016/j.brainres.2011.11.052>
57. András IE, Pu H, Tian J, Deli MA, Nath A, Hennig B, Toborek M, Andras IE, Pu H, Tian J, et al. Signaling mechanisms of HIV-1 Tat-induced alterations of claudin-5 expression in brain endothelial cells. *J Cereb Blood Flow & Metab* 2005; 25:1159-70; PMID:Can't; <http://dx.doi.org/10.1038/sj.jcbfm.9600115>
58. Pu H, Tian J, András IE, Hayashi K, Flora G, Hennig B, Toborek M, Andras IE, Hayashi K, Flora G, et al. HIV-1 Tat protein-induced alterations of ZO-1 expression are mediated by redox-regulated ERK 1/2 activation. *J Cereb Blood Flow Metab* 2005; 25:1325-35; PMID:15829913; <http://dx.doi.org/10.1038/sj.jcbfm.9600125>
59. Banerjee A, Zhang X, Manda KR, Banks WA, Ercal N. HIV proteins (gp120 and Tat) and methamphetamine in oxidative stress-induced damage in the brain: potential role of the thiol antioxidant N-acetylcysteine amide. *Free Radic Biol Med* 2010; 48:1388-98; PMID:20188164; <http://dx.doi.org/10.1016/j.freeradbiomed.2010.02.023>
60. Andras IE, Pu H, Deli MA, Nath A, Hennig B, Toborek M. HIV-1 Tat Protein Alters Tight Junction Protein Expression and Distribution in Cultured Brain Endothelial Cells. *J Neurosci Res* 2003; 74:255-65; PMID:14515355; <http://dx.doi.org/10.1002/jnr.10762>
61. Williams DW, Anastos K, Morgello S, Berman JW. JAM-A and ALCAM are therapeutic targets to inhibit diapedesis across the BBB of CD14+CD16+ monocytes in HIV-infected individuals. *J Leukoc Biol* 2015; 97:401-12; PMID:25420915; <http://dx.doi.org/10.1189/jlb.5A0714-347R>
62. Wu DT, Woodman SE, Weiss JM, McManus CM, D'Avessa TG, Hesselgesser J, Major EO, Nath A, Berman JW. Mechanisms of leukocyte trafficking into the CNS. *J Neurovirol* 2000; 6 Suppl 1:S82-5; PMID:10871769
63. Buckner CM, Luers AJ, Calderon TM, Eugenin EA, Berman JW. Neuroimmunity and the blood-brain

- barrier: Molecular regulation of leukocyte transmigration and viral entry into the nervous system with a focus on neuroAIDS. *J Neuroimmune Pharmacol* 2006; 1:160-81; PMID:18040782; <http://dx.doi.org/10.1007/s11481-006-9017-3>
64. 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; PMID:23922698; <http://dx.doi.org/10.1371/journal.pone.0069270>
65. 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; 32:143-50; PMID:22219277; <http://dx.doi.org/10.1523/JNEUROSCI.4266-11.2012>
66. Samikkannu T, Ranjith D, Rao KVK, Atluri VSR, Pimentel E, El-Hage N, Nair MPN. HIV-1 gp120 and morphine induced oxidative stress: role in cell cycle regulation. *Front Microbiol* 2015; 6:1-8; PMID:25653648; <http://dx.doi.org/10.3389/fmicb.2015.00614>
67. Ellaurie M, Calvelli TA, Rubinstein A. Immunodeficiency Virus (HIV) Circulating Immune Complexes in Infected Children. 1990; 6:1437-41; PMID:1981834
68. Banks WA, Akerstrom V, Kastin AJ. Adsorptive endocytosis mediates the passage of HIV-1 across the blood-brain barrier: evidence for a post-internalization coreceptor. *J Cell Sci* 1998; 111 (Pt 4):533-40; PMID:9443901
69. 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; 10:369-83; PMID:22591361; <http://dx.doi.org/10.2174/157016212802138724>
70. Ronaldson PT, Bendayan R. HIV-1 viral envelope glycoprotein gp120 produces oxidative stress and regulates the functional expression of multidrug resistance protein-1 (Mrp1) in glial cells. *J Neurochem* 2008; 106:1298-313; PMID:18485102; <http://dx.doi.org/10.1111/j.1471-4159.2008.05479.x>
71. Louboutin J-P, Strayer DS. Blood-Brain Barrier Abnormalities Caused by HIV-1 gp120: Mechanistic and Therapeutic Implications. *Sci World J* 2012; 2012:1-15; PMID:22448134; <http://dx.doi.org/10.1100/2012/482575>
72. Kanmogne GD, Kennedy RC, Grammas P. HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: possible pathway for HIV entry into the brain and HIV-associated dementia. *J Neuropathol Exp Neurol* 2002; 61:992-1000; PMID:12430716; <http://dx.doi.org/10.1093/jnen/61.11.992>
73. Shiu C, Barbier E, Cello F Di, Choi HJ, Stins M. HIV-1 gp120 as well as alcohol affect blood-brain barrier permeability and stress fiber formation: Involvement of reactive oxygen species. *Alcohol Clin Exp Res* 2007; 31:130-7; PMID:17207111; <http://dx.doi.org/10.1111/j.1530-0277.2006.00271.x>
74. Nakamura S, Endo H, Higashi Y, Kousaka A, Yamada H, Yano M, Kido H. Human immunodeficiency virus type 1 gp120-mediated disruption of tight junction proteins by induction of protease-mediated degradation of zonula occludens-1 and -2 in human brain microvascular endothelial cells. *J Neurovirol* 2008; 14:186-95; PMID:18569453; <http://dx.doi.org/10.1080/13550280801993630>
75. Louboutin J-P, Agrawal L, Reyes BAS, 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; 69:801-16; PMID:20613638; <http://dx.doi.org/10.1097/NEN.0b013e3181e8c96f>
76. Price TO, Ercal N, Nakaoka R, Banks WA. HIV-1 viral proteins gp120 and Tat induce oxidative stress in brain endothelial cells. *Brain Res* 2005; 1045:57-63; PMID:15910762; <http://dx.doi.org/10.1016/j.brainres.2005.03.031>
77. Annunziata P, Cioni C, Toneatto S, Paccagnini E. HIV-1 gp120 increases the permeability of rat brain endothelium cultures by a mechanism involving substance P. *AIDS* 1998; 12:2377-85; PMID:9875575; <http://dx.doi.org/10.1097/00002030-199818000-00006>
78. Cioni C, Annunziata P. Circulating gp120 alters the blood-brain barrier permeability in HIV-1 gp120 transgenic mice. *Neurosci Lett* 2002; 330:299-301; PMID:12270651; [http://dx.doi.org/10.1016/S0304-3940\(02\)00814-5](http://dx.doi.org/10.1016/S0304-3940(02)00814-5)
79. Toneatto S, Finco O, van der Putten H, Abrignani S, Annunziata P. Evidence of blood-brain barrier alteration and activation in HIV-1 gp120 transgenic mice. *AIDS* 1999; 13:2343-8; PMID:10597775; <http://dx.doi.org/10.1097/00002030-199912030-00005>
80. Kanmogne GD, Schall K, Leibhart J, Knipe B, Gendelman HE, Persidsky Y. HIV-1 gp120 compromises blood-brain barrier integrity and enhances monocyte migration across blood-brain barrier: implication for viral neuropathogenesis. *J Cereb Blood Flow Metab* 2007; 27:123-34; PMID:16685256; <http://dx.doi.org/10.1038/sj.jcbfm.9600330>
81. Kanmogne GD, Primeaux C, Grammas P. HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. *J Neuropathol Exp Neurol* 2005; 64:498-505; PMID:15977641; <http://dx.doi.org/10.1093/jnen/64.6.498>
82. Miller DS, Bauer B, Hartz AMS. Modulation of P-Glycoprotein at the Blood-Brain Barrier: Opportunities to Improve Central Nervous System Pharmacotherapy. *Pharmacol Rev* 2008; 60:196-209; PMID:18560012; <http://dx.doi.org/10.1124/pr.107.07109>
83. Miller DS. Pharmacology of the Blood Brain Barrier: Targeting CNS Disorders. 1st ed.; Elsevier Academic Press; c2014. Chapter 1, ABC Transporter Regulation by Signaling at the Blood-Brain Barrier: Relevance to Pharmacology; p. 1-24.
84. Ashraf T, Robillard KR, Chan GNY, Bendayan R. Role of CNS Transporters in the Pharmacotherapy of HIV-1 Associated Neurological Disorders. *Curr Pharm Des* 2014; 20:1543-63; PMID:23789949; <http://dx.doi.org/10.2174/13816128113199990464>
85. Lee CG, Ramachandra M, Jeang KT, Martin MA, Pastan I, Gottesman MM. Effect of ABC transporters on HIV-1 infection: inhibition of virus production by the MDR1 transporter. *FASEB J* 2000; 14:516-22; PMID:10698967
86. Ronaldson PT, Persidsky Y, Bendayan R. Regulation of ABC membrane transporters in glial cells: Relevance to the pharmacotherapy of brain HIV-1 infection. *Glia* 2008; 56:1711-35; PMID:18649402; <http://dx.doi.org/10.1002/glia.20725>
87. Polli JW, Jarrett JL, Stuenkel SD, Humphreys JE, Dennis SW, Brouwer KR, Woolley JL. Role of P-glycoprotein on the CNS disposition of amprenavir (141W94), an HIV protease inhibitor. *Pharm Res* 1999; 16:1206-12; PMID:10468021; <http://dx.doi.org/10.1023/A:1018941328702>
88. Edwards JE, Brouwer KR, McNamara PJ. GF120918, a P-glycoprotein modulator, increases the concentration of unbound amprenavir in the central nervous system in rats. *Antimicrob Agents Chemother* 2002; 46:2284-6; PMID:12069991; <http://dx.doi.org/10.1128/AAC.46.7.2284-2286.2002>
89. Kim RB, Fromm MF, Wandel C, Leake BF, Wood AJ, Roden DM, Wilkinson GR. The Drug Transporter P-glycoprotein Limits Oral Absorption and Brain Entry of HIV-1 Protease Inhibitors. *J Clin Invest* 1998; 101:289-94; PMID:9435299; <http://dx.doi.org/10.1172/JCI1269>
90. Toborek M, Lee YW, Flora G, Pu H, Andras IE, Wylegala E, Hennig B, Nath A. Mechanisms of the Blood-Brain Barrier Disruption in HIV-1 Infection. *Cell Mol Neurobiol* 2005; 25:181-99; PMID:15962513; <http://dx.doi.org/10.1007/s10571-004-1383-x>
91. Langford D, Grigorian A, Hurford R, Adame A, Ellis RJ, Hansen L, Masliah E. Altered P-glycoprotein expression in AIDS patients with HIV encephalitis. *J Neuropathol Exp Neurol* 2004; 63:1038-47; PMID:15535131; <http://dx.doi.org/10.1093/jnen/63.10.1038>
92. Hayashi K, Pu H, Tian J, Andras IE, Lee YW, Hennig B, Toborek M. HIV-Tat protein induces P-glycoprotein expression in brain microvascular endothelial cells. *J Neurochem* 2005; 93:1231-41; PMID:15934943; <http://dx.doi.org/10.1111/j.1471-4159.2005.03114.x>
93. Ashraf T, Ronaldson PT, Persidsky Y, Bendayan R. Regulation of P-glycoprotein by human immunodeficiency virus-1 in primary cultures of human fetal astrocytes. *J Neurosci Res* 2011; 89:1773-82; PMID:21826700; <http://dx.doi.org/10.1002/jnr.22720>
94. Ronaldson PT, Bendayan R. HIV-1 viral envelope glycoprotein gp120 triggers an inflammatory response in cultured rat astrocytes and regulates the functional expression of P-glycoprotein. *Mol Pharmacol* 2006; 70:1087-98; PMID:16790532; <http://dx.doi.org/10.1124/mol.106.025973>
95. Hayashi K, Pu H, Andras IE, Eum SY, Yamauchi A, Hennig B, Toborek M, Andras IE, Eum SY, Yamauchi A, et al. HIV-TAT protein upregulates expression of multidrug resistance protein 1 in the blood-brain barrier. *J Cereb Blood Flow Metab* 2005; 26:1052-65; PMID:16395283; <http://dx.doi.org/10.1038/sj.jcbfm.9600254>
96. Shawahna R. Physical and metabolic integrity of the blood-brain barrier in HIV infection: a special focus on intercellular junctions, influx and efflux transporters and metabolizing enzymes. *Curr Drug Metab* 2015; 16:105-23; PMID:26179607; <http://dx.doi.org/10.2174/138920021602150713114715>
97. Miller DS. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol Sci* 2010; 31:246-54; PMID:20417575; <http://dx.doi.org/10.1016/j.tips.2010.03.003>
98. Naik P, Cucullo L. In vitro blood-brain barrier models: Current and perspective technologies. *J Pharm Sci* 2012; 101:1337-54; PMID:22213383; <http://dx.doi.org/10.1002/jps.23022>
99. 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; PMID:21641584; <http://dx.doi.org/10.1016/j.brainres.2011.05.015>