Blood–Brain Barrier Properties of Human Immunodeficiency Virus Antiretrovirals

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Introduction

Human immunodeficiency virus (HIV) enters the central nervous system (CNS) early in infection and forms a reservoir in the brain as evidenced by the presence of large quantities of unintegrated viral DNA in the brains of HIV infected individuals.[1,2] The mechanism by which HIV enters the brain is not well understood, however, the resulting infection leads to a number of CNS disorders such as autoimmune deficiency syndrome (AIDS) dementia complex, HIV encephalitis, and peripheral neuropathy.[3,4] Current HIV therapies are focused on reducing the viral load in serum, however, the presence of HIV in sequestered compartments such as the brain significantly limits their efficacy. Hence, the ability of antiretrovirals to enter the brain in therapeutic amounts to treat HIV infection is an essential part of effective HIV therapy. The main barrier for transport of antiretroviral agents to the brain is the blood−brain barrier (BBB). To combat infection and inhibit the replication of HIV in the brain, antiretroviral agents must cross the BBB. Remarkably, there are very few studies that have addressed this issue. This review presents the current understanding of the BBB transport properties of the Food and Drug Administration (FDA)-approved HIV antiretrovirals. Future directions for a better understanding of the BBB permeability of current and upcoming HIV antiretrovirals to treat the devastating neurological and cognitive disorders seen in AIDS patients are also discussed.

Blood–Brain Barrier

The brain is protected by a unique regulatory barrier composed of brain microvessel endothelial cells (BMEC), astroglia, pericytes, perivascular macrophages, and basal lamina, which is collectively known as the BBB. The BMECs provide the functional and morphological basis of the BBB, which acts as a regulatory interface and limits the permeability of the brain to drugs. The selective permeability of the BBB is due to BMECs distinct morphological and enzymatic properties that enable them to form continuous and unfenestrated tight junctions with minimal endocytotic and pinocytotic activity. The presence of tight junctions limits the entry of many blood-borne elements, including small molecules and macromolecules as well as circulating leukocytes, to the brain.[5] The presence of efflux pumps such as P-glycoproteins, which are associated with multidrug resistance (MDR) in BMECs, also limits the transport of drugs across the BBB.

The Effect of HIV on the BBB

HIV infection of the brain is believed to occur through direct and indirect effects on BMECs of the BBB. For example, in vitro studies have shown that HIV readily infects human BMECs to allow its direct entry into the brain.[6] The indirect passage of HIV-infected monocytes or lymphocytes across the BBB via adhesion molecules of BMECs and astrocytes has also been reported.[6] Moreover, HIV entry to the brain may be facilitated by alterations in the BBB permeability due to elevated levels of circulating cytokines, such as TNFα and IL2, in infected patients.[7]

Only indirect methods have been used for determining changes in the integrity of the BBB. These methods include immunostaining for fibrinogen and immunoglobulin G, which are markers of vascular permeability, in postmortem brains, monitoring changes in the magnetic resonance imaging of the brain, and measuring cerebrospinal fluid (CSF) serum protein levels of the HIV-infected individuals. The results of immunostaining for immunoglobulin G and fibrinogen deposition in postmortem brains of AIDS and control patients to detect excess leakage across the BBB due to HIV infection have been inconclusive.[8] Histological, immunocytochemical, and ultrastructural analysis of brain tissues have not shown significant abnormalities either.[7] However, marked accumulations of serum proteins have been seen in the brains of patients with AIDS dementia.[7] Given the limited data and shortcoming of the methods used, the functional integrity of the BBB is thought to remain intact after HIV infection.[9]

HIV Antiretroviral Agents

Currently there are three classes of compounds available for treatment of HIV infection; these are nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors. NRTI inhibit HIV replication by blocking reverse transcription of the viral RNA and preventing the formation of DNA as a template for future viral replication. NNRTI inhibit HIV replication by binding through hydrophobic interactions to the reverse transcriptase catalytic sites. Protease inhibitors stop viral replication by inhibiting HIV encoded protease and result in the release of noninfectious immature viral particles. The generic name, brand/common

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Table 1—Physicochemical and Protein Binding Properties of HIV Antiretrovirals

generic name	brand/common names	MW	aqueous solubility (mg/mL)[a]	log D_{oct}[b]	% protein binding[a]
		nucleoside-inhibitors			
abacavir	Ziagen	286.3	77	1.20	~50[47]
didanosine	Videx, ddI	236.2	27.3	–0.54	<5
lamivudine	Epivir, 3TC	229.3	70	–0.92	<36
stavudine	Zerit, d4T	224.2	83	–0.72	negligible
zalcitabine	Hivid, ddC	211.3	76.4	–1.10	<4
zidovudine	Retrovir, AZT	267.2	20.1	–0.58	34–38
		non-nucleoside inhibitors			
delavirdine	Rescriptor	516.0	0.003	—	98
efavirenz	Sustiva	315.7	0.008[48]	—	99[26]
nevirapine	viramune	266.3	0.1	1.81	60
		protease inhibitors			
amprenavir	agenerase	505.2	0.04[49]	2.53	90[49]
indinavir	Crixivan	613.8	soluble	2.79	60
nelfinavir	Viracept	567.8	slightly soluble	4.0[50]	>98
ritonavir	Norvir	721.0	insoluble	—	99.3–99.5[51]
saquinavir	Invirase	670.7	insoluble	4.51	98

[a] Solubility in water at room temperature and protein binding as reported in the *Physician's Desk Reference*.[25] [b] Log D_{oct} = logarithm of octanol/phosphate buffered saline (pH 7.4) distribution coefficient.[11]

names, molecular weight, octanol/water distribution coefficients, and plasma protein binding of the approved HIV antiretroviral drugs are listed in Table 1.

The physicochemical properties of the currently marketed HIV antiretrovirals are vastly different. NRTIs are low molecular weight, highly water soluble, and (with the exception of the most recently approved NRTI, abacavir) very hydrophilic drugs. The extent of protein binding of NRTIs ranges from negligible for stavudine to ~50% for abacavir. Among the NNRTIs, nevirapine has significantly higher solubility and lower protein binding than either delavirdine or efavirenz. The latter two are almost completely protein bound. Protease inhibitors have higher molecular weights and are highly lipophilic compared with NRTIs and NNRTIs. Except for indinavir, protease inhibitors are highly protein bound.

Measuring BBB Penetration

In Vitro Studies—Isolation and culture of BMECs from the brain has led to the development of in vitro models for studying a variety of CNS drug delivery issues ranging from passive diffusion, carrier-mediated transport, and metabolism to specific factors affecting the BBB permeability.[10] In these models, BMECs can be grown as monolayers and retain the characteristics of brain endothelial cells in vivo, including the morphology, specific BBB enzyme markers, and tight intercellular junctions. The in vitro bovine BMEC monolayer permeability coefficients, P_{BMEC}, of different classes of HIV antiretroviral agents have recently been reported.[11,12] These agents included four NRTIs (didanosine, stavudine, zalcitabine, and zidovudine), two NNRTIs (nevirapine and delaviridine), and three protease inhibitors (amprenavir, indinavir, and saquinavir). Figure 1 shows P_{BMEC} values for these HIV antiretrovirals as well as for abacavir compared with those of progesterone, a highly permeable transcellular marker, and sucrose, a paracellular marker.[11,12]

The P_{BMEC} value for nevirapine is significantly higher than all the other HIV antireterovirals tested. The P_{BMEC} value for saquinavir is >2 orders of magnitude lower than nevirapine. The values for indinavir, amprenavir, and all

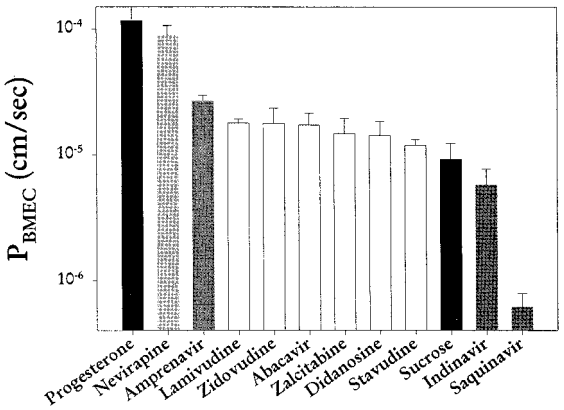

Figure 1—In vitro permeability of HIV antiretrovirals across the BMEC monolayers.[11,12]

NRTIs are also significantly lower than that of nevirapine. Moreover, the P_{BMEC} values for all NRTIs are not significantly different from each other because they have similar molecular weights and, with the exception of abacavir, very similar partitioning values. The low permeability of the protease inhibitors was attributed to their high lipophilicity and large molecular weights as well as higher potential for hydrogen bonding. The order of in vitro BBB permeability is: nevirapine ≫ amprenavir > abacavir, didanosine, stavudine, zalcitabine, and zidovudine > indinavir > saquinavir.[11,12]

In Vivo Studies—The concentration of antiretrovirals necessary to suppress HIV replication in the human brain is not known and naturally cannot be obtained in vivo. Hence, in vivo measurements of the extent of HIV antiretroviral penetration across the BBB in humans are limited to obtaining CSF concentrations from lumbar punctures. Clinical studies show that the presence of HIV antiretrovirals, such as stavudine and zidovudine, in CSF correlates well with improvement of HIV dementia.[13,14] HIV antiretroviral CSF concentrations have been most commonly used in conjunction with plasma concentrations to obtain the so-called CSF-to-plasma concentration ratios as a surrogate marker for the extent of the BBB permeability. These values have been reported with more frequency for the newly approved HIV antiretroviral inhibitors as a measure of their effectiveness in treating the CNS component of HIV infection (Table 2).

The major shortcomings of CSF-to-plasma concentration ratios as potential markers for drug penetration into the brain are the manner in which they are determined and interpreted. Direct comparisons cannot be made because of the varied experimental conditions (e.g., steady state versus non-steady state) under which these values have been obtained. Nor have these values been corrected for possible metabolism or protein binding in the brain. For example, the values for CSF-to-plasma concentration ratios reported for zidovudine are in the range of 0.15 to 1.35 and depend on the route of administration and dose given and hence do not conclusively determine how readily zidovudine crosses the BBB. Moreover, it has been shown that the CSF peak lags behind the plasma peak in concentration curves for zidovudine in plasma and CSF.[15] Hence, CSF-to-plasma concentration ratios are strongly time dependent and one time point measurements of CSF-to-plasma concentration ratios are not reliable. Furthermore, the absence of protein in CSF necessitates comparing CSF concentrations of HIV antiretrovirals to unbound plasma concentrations. Such comparisons are rarely done when CSF-to-plasma concentration ratios are reported.

The use of CSF levels may also be misleading because of differences in permeability of blood–CSF barrier (BCB)

Table 2—Cerebrospinal Fluid-to-Plasma and Brain-to-Plasma Concentration Ratios in Humans and Rat for Various HIV Antiretrovirals

antiretroviral	[CSF]/[plasma] in humans	[brain]/[plasma] in rat
	nucleoside-inhibitors	
abacavir	—	—
didanosine	0.16–0.19[52] not detected in CSF[55]	0.05, 0.007[41 a]
lamivudine	0.06–0.31[24]	—
stavudine	0.16–0.97[54]	0.33[55]
zalcitabine	0.09–0.37[24]	not measurable[56]
zidovudine	0.15–1.35[57]	0.19,[28] 0.23 ± 0.02[58]
	non-nucleoside inhibitors	
delavirdine	0.004[25]	—
efavirenz	0.01[26]	—
nevirapine	0.45[24]	1.0[24]
	protease-inhibitors	
amprenavir	—	—
indinavir	0.16,[28 b] 0.06[29]	0.18[33]
nelfinavir	not detected in CSF[32]	—
ritonavir	negligible[31]	—
saquinavir	negligible[30]	—

[a] Corrected for blood contribution to total brain concentration. [b] Mean CSF-to-plasma concentration ratios.

and the BBB.[9] The BBB is a more restrictive barrier than the BCB and because of its 5000-fold greater surface area, it is the main route of entry for drugs from blood to the brain.[9] Moreover, CSF and brain interstitial fluid are not in equilibrium and therefore the drug concentration in CSF does not necessarily reflect the extent of BBB transport of that drug because it may also include transport through the choroid plexus via the BCB. It then appears that CSF and CSF-to-plasma concentration ratios can only be used as qualitative measures of the ability of HIV antiretrovirals to cross the BBB and cannot be used to rank and order compounds in terms of their BBB permeability.

Due to inherent difficulty and indirect nature of the methods used in obtaining human data, a number of investigators have used laboratory animals to determine brain-to-plasma concentration ratios of HIV antiretrovirals. This parameter is the simplest and most direct measure of the BBB penetration of a given drug. The extent of brain drug uptake can also be presented by brain uptake index (BUI) or blood-to-brain transfer constant values.[16] The BUI is the extraction ratio of a drug relative to highly permeable compounds such as water or butanol. The major shortcomings of all of these values are that they are time dependent, require sacrificing of the animals, and must be corrected for the amount of drug retained in vascular space of the brain.[17] Furthermore, the relevance of these values in predicting the extent of penetration of the BBB in humans by HIV antiretroviral has not yet been established.

The reported in vivo studies of BBB properties of different classes of HIV antiretroviral agents are summarized next.

NRTI—There is no clear correlation between the BBB properties of NRTIs determined from animal studies and measurements of CSF values in humans. Available data on surrogate markers for BBB permeability, such as CSF-to-plasma concentration ratios in humans and brain-to-plasma concentration ratios in rats, suggest that the extent of BBB penetration for NRTIs is low and, in the case of stavudine and zidovudine, highly variable (Table 2). Moreover, clinical studies have shown that the BBB permeability of NRTIs could also be significantly different in humans. For example, in a small study of 16 patients, CSF HIV-1 RNA levels were reduced following zidovudine and not didanosine treatment.[18] Similarly, there were no clinical benefits associated with didanosine treatment suggesting that the extent of its BBB penetration in humans was also low.[19] The BBB permeability of zidovudine in humans is further supported by data suggesting reduction in the risk of developing AIDS dementia as well as improvement in cognitive impairment of AIDS patients after systemic treatment with zidovudine.[20,21] Lamivudine and stavudine also appear to penetrate the BBB in humans as indicated by a recent study where the levels of CSF HIV-1 RNA in 22 patients were reduced following treatment with these NRTIs.[22]

NNRTI—Nevirapine was the first NNRTI approved for treatment of HIV, and its BBB properties have been extensively studied.[11,12,23] The plasma-to-brain concentration ratios of nevirapine in rats and monkeys were shown to be close to 1.[23] In humans, nevirapine has a CSF-to-plasma concentration ratio of 0.45, which is approximately equal to the fraction not bound to plasma protein.[24] Taken together, these data support the contention that nevirapine readily crosses the BBB and enters the brain. In contrast, the penetration of delavirdine into the CNS appears to be low, as evidenced by its low CSF-to-plasma concentration ratio of 0.004.[25] Similarly, the BBB penetration of efavirenz is reported to be low, with a CSF-to-plasma concentration ratio of 0.01.[26]

Protease Inhibitors—In general, the extent of the BBB penetration of protease inhibitors has been reported as being low.[27–31] For example, CSF-to-plasma concentration ratios for indinavir are in the range of 0.06 to 0.16, and only negligible concentrations of ritonavir and saquinavir were present in CSF after oral administration.[27–31] Moreover, nelfinavir was not detected at all in CSF of patients following a variety of oral dosing regimens.[32] Limited BBB permeability of indinavir has also been observed in rats where a brain-to-plasma concentration ratio of 0.18 at steady state was reported.[33]

Combination Therapy—Currently, the recommended approach to HIV treatment is combination therapy with at least one protease inhibitor. Although protease inhibitors do not appear to cross the BBB to the extent of showing significant therapeutic efficacy, combinations of protease inhibitors (indinavir, ritonavir, or saquinavir) with zidovudine have been reported to prevent or reverse the progression of AIDS dementia, as suggested by brain magnetic resonance imaging studies.[34] Similarly, ritonavir and saquinavir, when taken in combination suppressed CSF HIV-1 RNA levels in 9 of 10 nucleoside-experienced patients, suggesting potential increases in the extent of their BBB penetration.[35] The addition of stavudine to this regimen increased the number of patients with undetectable HIV viral loads in serum and CSF to 80%.[36]

The effect of combination therapy on BBB permeability has only been reported in one in vitro study where there were no significant effects on the P_{BMEC} value for nevirapine in combination with amprenavir, delavirdine, didanosine, indinavir, saquinavir, stavudine, zalcitabine, or zidovudine.[11]

The Effect of Partitioning

It is generally accepted that the degree of lipophilicity of compounds is a determinant factor for their BBB permeability. For example, in vitro BBB permeability has been shown to correlate well with octanol/water partitioning and molecular weight for a variety of solutes.[37] The relationship between in vitro P_{BMEC} values and distribution coefficients (D) in pH 7.4 octanol/phosphate buffered solution for a variety of HIV antiretrovirals has been described as being roughly bell shaped.[11,12] Nevirapine appeared to have the optimum distribution coefficient ($\log D_{oct} = 1.81$) to allow for the highest permeability among the HIV

antiretrovirals studied. The permeability of hydrophilic NRTIs and very lipophilic protease inhibitors was significantly less than nevirapine. This relationship was descriptive only for the limited number of antiretroviral agents used in that study and was not meant to establish a general relationship between P_{BMEC} and D for all HIV antiretrovirals.[11]

The Effect of Efflux Pumps

P-Glycoprotein efflux pumps are present ubiquitously in both the BBB and the gastrointestinal tract.[38] A number of recent studies have shown that protease inhibitors, in general, are substrates for P-glycoprotein efflux pumps.[11,12,39,40] For example, it has been shown that indinavir, ritonavir, and saquinavir are substrates for these efflux pumps in BMECs and Caco-2 cells.[11,12,39] It has also been shown that the transports of indinavir, nelfinavir, and saquinavir in P-glycoprotein expressing cell lines L-MDR1 and Caco2 are affected by P-glycoprotein efflux pumps.[12,40] Similarly, the concentration of these protease inhibitors in the brain was shown to increase 7.4-, 10.6-, and 36.3-fold, respectively, in mice in which the MDR1a gene had been disrupted compared with the wild type.[40] This study clearly demonstrates the potetial effect of P-glycoprotein efflux pumps in reducing the BBB permeability of HIV protease inhibitors.

The effect of BBB efflux pumps on the limited distribution of NRTIs in the CNS after systemic administration is not clearly understood.[41−44] For example, microdialysis techniques in rabbits have shown that zidovudine is actively transported across the BBB and the BCB from brain to blood resulting in lower levels of zidovudine in brain and CSF than in plasma after systemic administration.[44] Similarly, it was shown that both zidovudine and didanosine were transported from the brain to the circulating blood across the BBB via saturable, probenicid sensitive efflux transporters.[45,46] However, in vitro transport studies using bovine BMECs could not identify selective efflux transport systems for NRTIs such as zidovudine.[11,12,43]

Future Directions

Understanding the mechanism of transport of HIV antiretrovirals across the BBB is essential for design and implementation of novel strategies for drug delivery to the brain to inhibit viral replication. The effect of HIV infection and the stage of the disease on the BBB properties and CNS uptake of drugs have not been fully understood. Further development of therapeutic strategies with the use of prodrugs, efflux inhibitors, and both in vitro and whole animals to evaluate intrinsic BMEC cellular permeability is needed. Although an HIV antiretroviral that is a substrate for BMEC efflux pumps may be taken alone with no adverse affects, the addition of other drugs that are competitive substrates for these pumps may affect the BBB permeability and efficacy of the first drug. Hence, the role of cellular efflux pumps on permeability of HIV antiretrovirals should be further studied to allow for rational drug delivery approaches for optimum BBB penetration.

This field of research is active and is likely to expand based on the paucity of information on the BBB permeability of currently used HIV antiretrovirals. Moreover, the BBB properties of novel classes of HIV antiretrovirals, such as HIV fusion and integrase inhibitors, need to be evaluated to determine their overall effectiveness in HIV therapy.

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