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Review Article

Theme: Celebrating Women in the Pharmaceutical Sciences
Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

HIV-1 Sanctuary Sites—the Role of Membrane-Associated Drug Transporters and Drug Metabolic Enzymes

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Abstract. Despite significant advances in the treatment of human immunodeficiency virus-1 (HIV) infection with highly active antiretroviral drug therapy, the persistence of the virus in cellular and anatomic reservoirs is a major obstacle preventing total HIV eradication. Viral persistence could result from a variety of contributing factors including, but not limited to, non-adherence to treatment and adverse drug reactions, latently infected cells carrying replication-competent virus, drug–drug interactions, and inadequate antiretroviral drug (ARV) concentrations reached in several anatomic sites such as the brain, testis, and gut-associated lymphoid tissues. The distribution of ARVs at specific sites of infection is primarily dependent on drug physicochemical properties and drug plasma protein binding, as well as drug efflux, influx, and metabolic processes. A thorough understanding of the functional roles of drug transporters and metabolic enzymes in the disposition of ARVs in immune cell types and tissues that are characterized as HIV reservoirs and sanctuaries is critical to overcome the challenge of suboptimal drug distribution at sites of persistent HIV infection. This review summarizes the current knowledge related to the expression and function of drug transporters and metabolic enzymes in HIV cellular and anatomic reservoirs, and their potential contribution to drug–drug interactions and insufficient drug concentration at these sites.

KEY WORDS: antiretroviral drugs; ATP-binding cassette transporters; drug metabolic enzymes; HIV reservoirs; HIV sanctuaries.

INTRODUCTION

Human immunodeficiency virus-1 (HIV) infection remains a global challenge, which resulted in about 38 million people living with the virus in 2019 (1). The number of people living with HIV and accessing antiretroviral therapy (ART) has increased substantially to about 25.4 million in 2019 (1). However, the total number of people living with HIV continues to rise and many people are still dying from AIDS-related causes each year (1). ART has been effective in suppressing plasma viral loads, reducing HIV-associated mortality, and improving the quality of life of people living with HIV. However, in addition to persistent infection in viral reservoirs and sanctuary sites, there are several contributing factors that could also prevent HIV eradication. These include non-adherence to therapy and the emergence of drug-resistant HIV strains, lack of a preventative or therapeutic vaccine, and possible socioeconomic challenges in low- to middle-income countries. In addition to the pill burden, the drug toxicity and adverse drug events associated with some ARVs could decrease adherence to ART (2). Poor adherence to ART increases the risk of incomplete viral suppression and the emergence and transmission of drug-resistant HIV strains (3,4). Furthermore, low drug penetration in HIV sanctuary sites such as the brain and male genital tract has been associated with the development of drug-resistant virus (5–7). There have also been many challenges in the development of an effective therapeutic and/or preventative HIV vaccine (8), indicating the need for further advances in this research field. Moreover, socioeconomic obstacles including lack of access to treatment and healthcare in low- to middle-income countries must be overcome to achieve full HIV eradication. As the prevalence of HIV increases with the use of ART (1), the cost of lifetime access to treatment and healthcare also increases and could become an economic burden in these countries where the pandemic is severe (9).

Persistent infection in viral reservoirs and sanctuary sites remains one of the greatest obstacles impeding HIV eradication. Suboptimal penetration of ARVs in HIV viral reservoirs and sanctuary sites could contribute to ineffective ART

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treatment and persistent infection. In addition to drug physicochemical properties, membrane-associated drug transport proteins belonging to the ATP-binding cassette (ABC) and solute carrier (SLC) superfamilies of membrane transporters, as well as drug metabolic enzymes, play an important role in regulating ARV disposition in mammalian tissues. In this review, we discuss factors that could contribute to low ARV concentrations in cellular and anatomic HIV reservoirs; particularly, we address the role of drug transporters and metabolic enzymes in HIV pharmacotherapy and their potential contribution to persistent HIV infection.

Antiretroviral Therapy

The use of ART has effectively reduced viral load and decreased HIV-related mortality and morbidity. However, the existence of viral reservoirs, limited ARV drug penetration in sanctuary sites, and drug–drug interactions which could lead to treatment failure or drug-induced toxicities are some of the challenges preventing complete viral suppression (10,11). ART for treatment-naïve patients usually consists of two nucleoside reverse transcriptase inhibitors (NRTIs) administered in combination with a third drug from one of three drug classes: an integrase strand transfer inhibitor (INSTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI), or a protease inhibitor (PI) boosted with a pharmacokinetic enhancer (cobicistat or ritonavir) (12). The current recommended regimens are those including the INSTIs dolutegravir, raltegravir, and bictegravir, or PIs such as darunavir and atazanavir in certain clinical situations (12). ARVs are classified based on the stages of the HIV life cycle which they target. CCR5 antagonists prevent virus attachment to the membrane receptors of the host cell, while fusion inhibitors interfere with membrane fusion which normally allows the insertion of the viral genome into the host cell (13). Both NRTIs and NNRTIs interrupt the activity of viral reverse transcriptase and prevent the virus from converting its RNA into DNA. INSTIs block the integration of the viral DNA into the host cell's DNA chromosome, and PIs inhibit the protease enzyme resulting in the release of structurally disorganized and non-infectious viral particles (13).

HIV Persistence in Viral Reservoirs and Sanctuary Sites

As previously indicated, a major challenge in the pharmacological treatment of HIV infection is the persistence of the virus in viral reservoirs and sanctuary sites. The use of the terms "reservoirs" and "sanctuary sites" can be inconsistent in the literature. For the purpose of this review, and based on definitions by others, a viral reservoir is a cell type or an anatomical compartment in which replication-competent forms of the virus persist with more stable kinetic properties than the main pool of actively replicating virus (14,15). The reservoir is further classified as latent (i.e., transcriptionally silent) if the infected cells are not producing virus but maintain the capacity to do so upon stimulation or discontinuation of ART (15). A viral sanctuary site is an anatomical compartment where HIV can replicate during ART due to poor penetration of ARVs and/or due to special biological properties such as that site being immunoprivileged (10). Anatomical sites such as the brain may be described as both a reservoir due to long-lived/non-productively infected cells and a sanctuary site due to limited drug penetration (16). The mechanisms underlying HIV reservoir formation have not been fully elucidated. However, a primary cause for the virus to persist during ART is the residual viral replication which may not be fully suppressed in drug-privileged anatomical compartments (14). The persistence of a small pool of cells carrying silent integrated genomes may also exist. These cells can be reactivated and reignite the infection. Furthermore, persistent immune dysfunctions may fail at controlling residual replication and reactivation from latently infected cells (14).

One of the best characterized cellular reservoirs for HIV in virally suppressed individuals on ART are CD4+ T cells (17). Additionally, non-conventional HIV cell reservoirs have been described, including CD8+ T cells (18), the CD3+CD4−CD8− double-negative T cell subset (19), and cells from the myeloid lineage including circulating monocytes, macrophages, and dendritic cells (17), as well as astrocytes, microglia, and perivascular macrophages in the central nervous system (CNS) (20).

Tissue sites such as the gut-associated lymphoid tissue (GALT), the male and female genitourinary tracts, and the CNS are important sources of persistent infection (Fig. 1a), and are also recognized as viral sanctuaries which demonstrate limited ARV penetration (21–23). Decreased drug concentrations in mononuclear cells isolated from GALT compared to peripheral blood mononuclear cells (PBMCs) have been demonstrated (11). Furthermore, drug concentrations in plasma or PBMCs did not predict those in lymphoid compartments, where most viral replication occurs and persists (11). The testis has been identified as a distinct anatomic HIV reservoir in ART-treated individuals (24). Our group reported low ARV concentrations in testicular tissue of individuals living with HIV on ART (22), as well as demonstrated reduced permeability of ARVs at the mouse blood–testis barrier (BTB) formed by epithelial Sertoli cells (25,26). The CNS is protected by the blood–brain barrier (BBB) composed of microvessel endothelial cells, which isolates the brain from the circulating blood and limits the permeability of compounds including ARVs into the brain parenchyma (27). Furthermore, the blood–cerebrospinal fluid barrier (BCSFB), composed of the epithelium of the choroid plexus, restricts the passage of molecules and cells into the cerebrospinal fluid (28). The presence of these two barriers provides an obstacle to effective ARV penetration, which could potentially result in CNS viral replication (15).

CAN LOW ARV CONCENTRATIONS CONTRIBUTE TO HIV PERSISTENCE IN VIRAL RESERVOIRS AND SANCTUARIES?

HIV is known to persist in anatomic sites such as the GALT, the male and female genitourinary tracts, and the CNS which display limited ARV penetration (21,23,24,29). Several studies demonstrating that insufficient ARV intracellular concentrations could be associated with continuous HIV replication in tissue sanctuaries are discussed below.

The Central Nervous System

HIV can enter the CNS during early infection and target cells such as perivascular macrophages, microglia, and astrocytes. These are long-lived cells which are capable of harboring replication-competent integrated provirus, thus enabling the virus

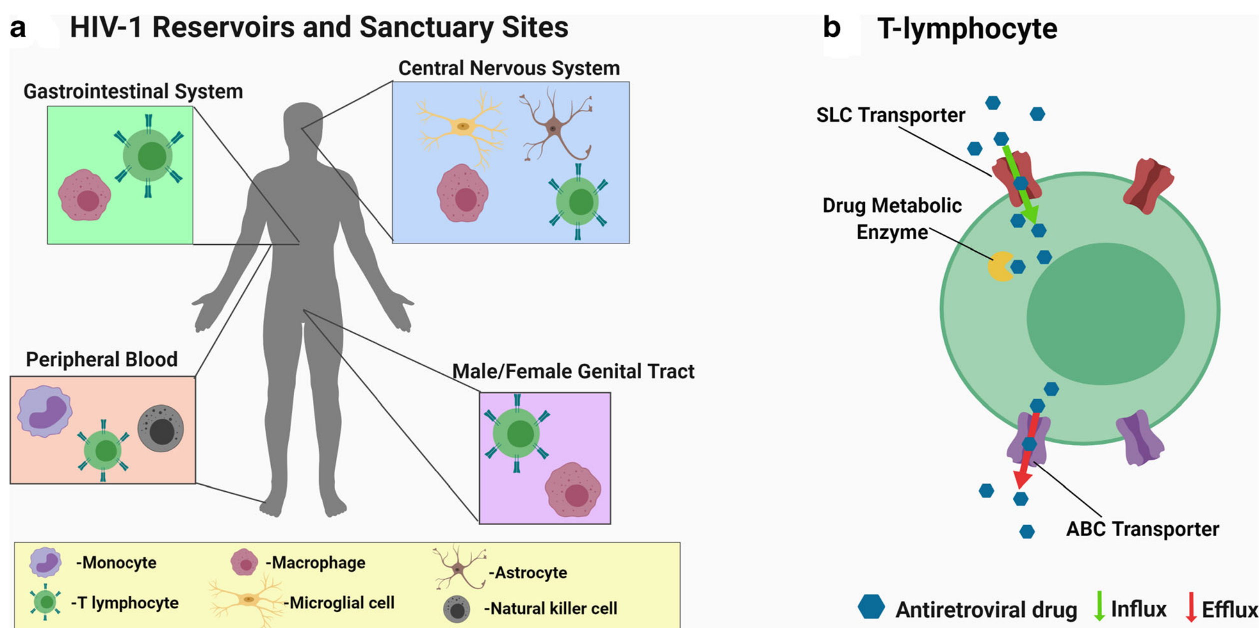

Fig. 1. Drug transporters, metabolic enzymes, and HIV reservoirs. **a** Illustration of several proposed cellular and anatomic HIV reservoirs. **b** Drug transporters and metabolic enzymes functionally expressed on a T lymphocyte representing the HIV reservoir. SLC transporters may facilitate the uptake (influx) of an antiretroviral drug across the cell membrane into the intracellular compartment, where it is metabolized by phase I/II drug metabolic enzymes or extruded (efflux) to the extracellular compartment by ABC transporters, thereby reducing its intracellular concentration. Figure created using BioRender

to persist for long periods (20). Additionally, low permeability of ARVs across the BBB and the BCSFB could permit ongoing HIV replication in the CNS despite viral suppression in the periphery. Letendre *et al.* demonstrated that ARVs with poorer penetration into the brain was associated with continued HIV replication in the CNS as indicated by higher CSF HIV viral loads (30). Work by Gray *et al.* further demonstrated that ARVs with high CNS penetrating effectiveness scores could also have reduced efficacy in astrocytes. Particularly, lamivudine and zidovudine demonstrated insufficient HIV inhibitory activity in astrocytes with EC_{90} values 187- and 110-fold above achievable CSF concentrations (31).

Asahchop *et al.* investigated the comparative antiviral activity of several ARVs in human fetal microglia, bone marrow-derived macrophages, and activated peripheral blood mononuclear cells (PBMCs); following *in vitro* HIV infection and ARV treatments, darunavir, etravirine, zidovudine, and raltegravir displayed significantly higher EC_{50} (ng/ml) values against HIV in human fetal microglia (8.1, 3.6, 27, 3.3, respectively) compared to PBMCs (1.2, 0.6, 2.1, 1.1, respectively), suggesting that these drugs were less effective in controlling viral replication in the brain compartment (32). In parallel, HIV-RNA and DNA with associated neuroinflammatory responses were detected in brain tissues collected from virally suppressed patients within hours of their last ARV doses (32). Together, these data suggest that the use of ARVs with suboptimal penetration in the brain could lead to low intracellular drug concentrations and persistent infection in brain cells (32).

The Gastrointestinal System

The GALT represents a highly susceptible target for HIV infection and enhances HIV replication. Detectable levels of HIV replication have been reported in tissue biopsy samples from GALT during ART. Chun *et al.* found higher frequencies of HIV-infected cells in GALT when compared to PBMCs and further demonstrated cross-infection between these compartments during ART, which suggests ongoing viral replication in the GALT (21). Fletcher *et al.* demonstrated that inadequate penetration of ARVs into GALT compartments allowed ongoing low-level replication sufficient to maintain a state of immune activation (11). Their results demonstrated that, in individuals with mean therapeutic plasma concentrations of ARVs such as atazanavir (377 ng/ml), darunavir (1310 ng/ml), ritonavir (56.5 ng/ml), and efavirenz (1750 ng/ml), intracellular concentrations were lower in mononuclear cells isolated from the ileum, rectum, and lymph nodes, compared to PBMCs. Specifically, atazanavir, darunavir, and efavirenz demonstrated average concentrations that were 100%, 99%, and 94% lower in lymph node mononuclear cells compared to PBMCs, respectively (11). They also showed that four out of the twelve individuals enrolled in the study had changes in their rate of reduction of the follicular dendritic cell network pool which reflected continuous viral production during ART, and correlated with lower ARV drug levels in these tissues over six months of therapy (11).

Lorenzo-Redondo *et al.* performed deep sequencing, phylogenetic analysis, and mathematical modeling to characterize the temporal structure and divergence of viral sequences from blood and inguinal lymphoid tissue compartments collected from three subjects up to 6 months after treatment. Their model predicted that the virus, predominantly the drug-sensitive rather than the drug-resistant strain, could proliferate in the lymphoid tissue sanctuary site where drug concentrations were low (33).

Their calculations also demonstrated that increasing drug effectiveness or penetration in the spatial model of the sanctuary site could lead to the emergence of drug-resistant strains or the elimination of ongoing replication (33). Overall, the temporally and compartmentally structured sequence data suggest that continuous virus production from infected cells in the lymphoid tissue sanctuary, where drug concentrations are limited, could continue to replenish the viral reservoir.

More recently, Estes *et al.* demonstrated continuous viral replication in lymphatic tissues obtained from SIV-infected macaques and people living with HIV, despite suppressive ART in plasma. Compared to PBMCs, they found lower intracellular ARV concentrations in lymph nodes, GALT, and rectal-associated mucosal tissue from SIV-infected macaques, which correlated with low-level virus production in these tissues (34). The median intracellular concentration ratios reported were 0.4 for tenofovir diphosphate and 0.3 for emtricitabine triphosphate in GALT compared to PBMCs of six SIV-infected macaques, while darunavir was below quantifiable limits (34). Overall, these results support the hypothesis that lower tissue ARV concentrations could contribute to persistent HIV infection (34). Suboptimal ARV penetration into tissue and cell reservoirs could potentially be one of the mechanisms enabling HIV replication and spread throughout the body during therapy.

The Male Genital Tract

A number of studies have demonstrated evidence of HIV in immune cells infiltrating tissues along the male genital tract including the epididymis, prostate, and seminal vesicles; see review cited (35). In addition, the CD4 receptor is detectable on T cells and macrophages infiltrating the testicular interstitium (24,36). A subset of individuals receiving ART continues to have detectable levels of HIV RNA in genital tract secretions despite undetectable levels in blood and lack of other sexually transmitted infections (37). Osborne *et al.* provided evidence that an intensified ART regimen reduced, but did not totally prevent, high-level HIV shedding in semen (37). Data from the Orchid studies demonstrated the testis as a distinctive anatomical reservoir for HIV persistence (24,38). Jenabian *et al.* showed that total HIV DNA was detected in at least one testis from each of the six participants who were on suppressive ART, while five participants demonstrated integrated HIV DNA. Furthermore, an increase in effector-memory T cell subsets from HIV-infected and uninfected testicular tissues, compared to matched PBMCs, and a significant increase in CCR5 expression on testicular T cells were observed (24). Recently, HIV diversity and compartmentalization were assessed using genetic and phylogenetic analyses in testicular tissue and blood obtained from 10 individuals on suppressive therapy (38). Following evaluation of intact HIV *nef* sequences, 60% of the participants displayed significant blood-testis genetic compartmentalization which was attributed to clonal expansion of HIV-infected cells, suggesting that the testis is a distinct site of persistent HIV infection (38). In addition, our group measured ARV concentrations in the testicular tissue of individuals living with HIV and found that relative to plasma concentrations, lower accumulation, below the therapeutic range, of efavirenz (25%) and darunavir (19%) was consistently displayed (22). In contrast, emtricitabine (117%), lamivudine (112%), and tenofovir (85%) penetrated effectively into the testicular tissue (22). Previous studies quantifying ARV concentrations in seminal plasma also reported high accumulation of emtricitabine (440%), lamivudine (420%), and tenofovir (510%) and poor accumulation of efavirenz (9%) and darunavir (11%) relative to blood plasma (39), similar trends to what we observed in testicular tissue. Overall, the male genital tract serves as a site of (i) persistent HIV infection displaying viral compartmentalization in several of its tissues, (ii) isolated HIV shedding in semen, and (iii) limited penetration of several ARVs, particularly in the testes.

The Female Genital Tract

HIV can enter the female genital tract through the mucosal epithelium of the vagina, ectocervix, or endocervix (40). The virus can then access and infect CD4+ T cells, macrophages, and dendritic cells between the leaky ectocervical and/or vaginal epithelium in the lower genital tract (40). In addition, the endocervical columnar epithelium may facilitate HIV entry via endosomal transcytosis (40). Lymphocytes from both vaginal and ectocervical mucosa can retain a high level of HIV replication (40). Limited penetration of PIs and NNRTIs has been reported in the cervicovaginal fluid of women receiving suppressive ART. In a study by Kwara *et al.*, lower concentrations of fosamprenavir (54.8 ng/ml *vs*. 1009.9 ng/ml), nelfinavir (113.7 ng/ml *vs*. 2363.8 ng/ml), atazanavir (390.2 ng/ml *vs*. 1188 ng/ml), efavirenz (18.4 ng/ml *vs*. 2087.8 ng/ml), and nevirapine (272 ng/ml *vs*. 3499.8 ng/ml) in cervicovaginal fluid compared to blood plasma were reported (41). Another study by Dumond *et al.* also demonstrated lower concentrations of lopinavir and atazanavir, after the first dose and at steady state, in cervicovaginal fluid compared to blood plasma (42). HIV target cells including CD4+ T cells and other cell types expressing the major coreceptors, CCR5 and CXCR4, are present in the upper and lower female reproductive tract and are susceptible to HIV infection (23). Furthermore, HIV shedding in the female genital tract has been reported in women on ART despite plasma viral suppression (43). Together, these studies highlight the need for therapeutic strategies to overcome the various factors that could contribute to low and/or ineffective ARV penetration at sites of HIV infection.

FACTORS CONTRIBUTING TO LOW ARV CONCENTRATIONS

The extent of ARV distribution into mammalian tissues is primarily dependent on the physicochemical properties of the drugs (i.e., molecular weight and size, lipophilicity, and ionization) and the extent of drug plasma protein binding, as well as a dynamic interplay between drug efflux, influx, and metabolic processes involving drug transporters and metabolic enzymes.

Physicochemical Properties of the Drug

The diffusion of a drug through a cell membrane is primarily determined by its molecular weight, lipophilicity, and the degree of ionization. Compounds with molecular weight below 500 Da are usually classified as low molecular weight and can more favorably diffuse across biological cell membranes. In general, increased molecular weight has been associated with decreased solubility and decreased membrane permeability (44). The partition coefficient of a compound between water and a lipophilic solvent such as *n*-octanol is a useful tool that can help to predict how well a drug will diffuse across the cell membrane. The higher the partition coefficient, the greater the lipophilicity of the drug and its ability to passively diffuse intracellularly. Glynn and Yazdanian studied the partition coefficient (referred to as distribution coefficient, Log *D*, in the study) between octanol and phosphate-buffered saline of several ARVs including didanosine, zidovudine, amprenavir, nevirapine, indinavir, and saquinavir using a bovine BBB model (45). They found that nevirapine was most permeable and moderately lipophilic with a partition coefficient of 1.8, while the NRTIs were more hydrophilic and less permeable, even though they were similar in size and molecular weight to nevirapine. The PIs indinavir and saquinavir were more lipophilic and had higher partition coefficients of 2.8 and 4.5, respectively, compared to all the other ARVs in this study (45). However, PIs serve as substrates for several membrane-associated drug efflux transport proteins which could significantly limit their ability to permeate across cell membranes.

Cell membranes are usually impermeable to the ionized forms of drugs; therefore, the concentration of a drug that is available for passive diffusion is dependent on the ionization of the molecule and its dissociation constant (pKa), as well as the pH of its environment (46). The degree of ionization for acidic and basic drugs could determine their membrane permeability. Weakly acidic drugs exist in a more un-ionized form at lower pH which increases their membrane permeability. On the contrary, weakly basic drugs exist in a more ionized form at lower pH, or will be mainly un-ionized in a basic environment (46,47). Therefore, ARVs such as nevirapine (pKa 2.8), saquinavir (pKa 1.1, 7.1), and indinavir (pKa 6.2) are weak bases which are more likely to cross lipid membranes when the environment is at physiological pH of 7.4 (46–48). Nevirapine is largely un-ionized in plasma (pH 7.4), which may also contribute to its high CNS penetration (47).

Extent of Protein Binding in Blood Plasma, Cerebrospinal Fluid, and Seminal Plasma

From the systemic circulation, a drug is distributed into tissues based on its relative affinity for tissue versus blood plasma components. Many drugs bind to circulating proteins such as albumin, acid glycoproteins, globulins, and lipoproteins (49). In general, PIs are lipophilic organic bases which bind more than 90% to α1-acid glycoprotein, except for indinavir. NNRTIs usually bind to albumin. Specifically, efavirenz displays high protein binding of over 99%, while nevirapine demonstrates 60% protein binding to albumin (49). Only the unbound (free) fraction of the drugs in plasma can cross the cell membrane to access HIV viral enzymes in infected cells (49). It is important to note that protein binding may not be directly related to the cellular accumulation of some drugs. For example, indinavir displays a higher free fraction in blood plasma but accumulates to a lower level inside lymphocytes when compared to other PIs (46). Data on whether protein binding of ARVs can affect their antiviral activity is very limited. *In vitro* experiments have demonstrated that adding physiologic concentrations of α1-acid glycoprotein into the cell medium reduces the concentration of the unbound fraction of drugs such as saquinavir, ritonavir, and indinavir (50). Physiologically, an equilibrium must be reached between the concentration of the unbound drug in plasma and tissue. However, an exception can be in cells or tissues that express active efflux mechanisms such as the BBB and the BTB. As such, these compartments generally display lower concentrations of ARVs, especially PIs, compared to blood plasma (49).

Molecular weight greater than 500 Da and protein binding over 90% usually impede drug membrane permeability (51). NRTIs such as zidovudine, lamivudine, and abacavir have low protein binding and molecular weights less than 500 Da which allow for better CNS penetration (47). On the other hand, PIs have high molecular weights over 500 Da, plasma protein binding over 90%, and are substrates for drug efflux transporters which may contribute to their low penetration in the CNS and other tissues. Molecular weight and protein binding do not solely predict CNS penetration. For example, tenofovir has a low molecular weight and protein binding; however, this drug was reported to have low CSF concentration and CNS penetration (52). The protein concentration in the CSF is usually lower than that in blood plasma; albumin ranges from 7.8 to 40 mg/l in CSF and 33–55 g/l in blood plasma. This, along with low drug concentrations, can make it challenging to measure unbound CSF drug concentrations (53). Drugs with protein binding lower than 90% usually display effective concentration in the seminal plasma, suggesting that diffusion is the primary mechanism for drug transport to the lumen of the male genital tract. Weakly basic, lipophilic compounds with minimal plasma protein binding and high free drug concentrations are expected to achieve higher seminal plasma concentrations. Hence, many PIs with high protein binding in blood plasma have low seminal plasma concentrations, while NRTIs which have lower protein binding display high seminal plasma concentrations (48). In addition to physicochemical and protein binding properties, the distribution of a drug also depends on its interactions with drug transporters and metabolic enzymes, which play a crucial role in regulating effective intracellular concentrations.

Drug Transporters and Metabolic Enzymes

The disposition of ARVs, like many other drugs, involves influx into or efflux out of cells by the solute carrier (SLC) and ATP-binding cassette (ABC) superfamilies of biological membrane-associated drug transport proteins, respectively (Fig. 1b). The ABC superfamily includes several drug efflux transporters such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP) (54). ARVs also interact with several SLC transporters including organic anion-transporting

polypeptides and organic anion and organic cation transporters, as well as equilibrative and concentrative nucleoside transporters (54). SLC transporters primarily transport drug molecules intracellularly; therefore, inhibition of their functional activities could contribute to lower intracellular concentrations or drug interactions of ARV substrates (54,55). Drug metabolic enzymes facilitate the conversion of drugs to hydrophilic molecules that can be more favorably eliminated by biliary or urinary excretion (56). The metabolism of a drug usually leads to its inactivation, although in some cases, it can result in the formation of an active metabolite (56). Many ARVs can act as substrates, inducers or inhibitors of drug transporters and metabolic enzymes (Table I) and could result in complex drug interactions and/or inadequate drug distribution in HIV tissue sanctuaries and cellular reservoirs (55). The next section of this review will focus on the expression and function of drug transporters and metabolic enzymes in HIV tissue sanctuaries and cellular reservoirs, where they could play a role in reducing intracellular ARV concentrations.

EXPRESSION AND/OR FUNCTION OF DRUG TRANSPORTERS AND METABOLIC ENZYMES IN CELLULAR AND ANATOMIC HIV RESERVOIRS

SLC Transporters

SLC transporters are primarily involved in the cellular uptake of a wide range of drugs including ARVs (91). Our group and others have demonstrated the roles of these transporters in regulating the intracellular permeability of numerous ARVs across biological membranes (92–95). We refer the reader to previous articles from our group (54,55) for extensive review on SLC transporters and ARV disposition. In this review, we will provide a detailed discussion on the functional expression of ABC drug efflux transporters and metabolic enzymes in HIV reservoirs and sanctuary sites, due to their roles in reducing intracellular ARV concentrations.

ABC Transporters

ABC transporters are primary active membrane-associated transport proteins which directly use energy from ATP hydrolysis to actively efflux molecules, including drugs, extracellularly against a concentration gradient (96). ABC transporters such as P-gp, BCRP, and MRPs are known to play a key role in multi-drug resistance (96). P-gp is widely distributed in mammalian tissues and is capable of transporting all PIs, and several INSTIs, NRTIs, and NNRTIs (54). BCRP is involved in the transport of NRTIs including abacavir, zidovudine, stavudine, lamivudine, and didanosine (57). Studies have also shown in vitro that the INSTIs raltegravir and dolutegravir are substrates of BCRP (58,59). MRPs also play an important role in ARV permeability. MRP1 and MRP2 were shown to be involved in the efflux of PIs such as ritonavir, lopinavir, atazanavir, nelfinavir, saquinavir, and indinavir in vitro (97,98), while tenofovir is a substrate of MRP4 (60), see Table I.

ARV Efflux Transporters in the CNS

ABC transporters expressed at the BBB and BCSF barriers can regulate the permeability of endogenous substances and xenobiotics including ARVs into and out of the CNS (99). Furthermore, perivascular macrophages, microglial cells, and astrocytes are known to express several ABC drug efflux transporters which could restrict intracellular ARV penetration in critical HIV cellular targets (27). Our group was the first to demonstrate localization of P-gp along the plasma membrane and nuclear envelope of a continuous rat microglia cell line (MLS-9) and further confirmed the functional activity of this transporter in the context of HIV pharmacotherapy in the same cell line (27,100). P-gp localization was also demonstrated *in situ* in capillary endothelial cells, astrocytes, and pericytes of human and rat brain tissues (101). We also confirmed the functional expression of MRP1, MRP4, and MRP5 in the MLS-9 cell line and primary cultures of rat microglia (102,103). In addition, protein expression and localization of MRP1, MRP4, and MRP5 have been identified in human astrocytes (104,105). The mRNA and protein expression of BCRP was reported in a mouse microglia BV-2 cell line system (106), as well as in primary cultures of rat astrocytes by our group (107). Furthermore, our group and others have shown that HIV viral proteins such as gp120 and Tat could alter the function and expression of transporters such as P-gp and MRP1 at the human and/or rodent BBB, as well as in brain astrocytes (108–112). Treatment of HIV infection in the brain can be challenging as several ARVs display poor CNS permeability (52). Drug efflux transporters present in glial cells and at the BBB may limit the overall accumulation of ARVs in the CNS.

ARV Efflux Transporters in the Gastrointestinal Tract

Expression of P-gp, BCRP, and MRP1–5 was demonstrated in colorectal CD4+ T cells, with MRP3, MRP5, and BCRP demonstrating significantly higher expression in colorectal CD4+ T cells compared to circulating CD4+ T cells (113). In addition, their expression along the gastrointestinal epithelium can regulate the absorption of orally administered ARVs. The functional expression of drug transporters and interactions with ARVs at the blood–intestinal mucosa barrier has been investigated by our group (54,55,93,95). We also previously characterized the expression of drug transporters in the upper and lower gastrointestinal tract and evaluated the effects of HIV infection and ART on these proteins (114,115). These studies demonstrated downregulation of the expression of transporters such as P-gp, BCRP, and MRP2 in upper gastrointestinal tissue biopsies and recto-sigmoid colon biopsies of ART-naïve individuals living with HIV, while their expression levels were either at baseline or higher in ART-treated patients. It has been demonstrated that HIV infection or ART could alter the expression of drug efflux transporters. In the context of HIV-associated inflammation, downregulation of ABC transporters has been attributed to the activation of transcription factors such as NF-kB by proinflammatory cytokines that can ultimately modulate their gene expression (110). On the other hand, as discussed in "ARV-Mediated Regulation of Drug Efflux Transporters and Metabolic Enzymes Via Nuclear Receptors," interactions between ARVs and specific transcription factors could result in the upregulation of the transporters as observed in ART-treated individuals.

Table I. Interactions of Drug Efflux Transporters and Metabolic Enzymes with ARVs

ARV class	ARVs[a]	Substrate (K_m)	Inhibitor (IC_{50})	Inducer (fold-increase)	References
Integrase inhibitors	Dolutegravir	P-gp BCRP CYP3A4 UGT1A1 (21 μM)			(58,66)
	Raltegravir	P-gp BCRP UGT1A1 (52 μM)			(59,67)
	Bictegravir	CYP3A4 UGT1A1			(62)
	Elvitegravir	P-gp CYP3A4 (68 μM) UGT1A1	P-gp (17.7 μM)[b] BCRP (15.7 μM)		(61,68)
Nucleoside reverse transcriptase inhibitors	Lamivudine	BCRP	P-gp MRP1–3 (≥ 200 μM)	P-gp (2x, 500 μM)[c]	(57,69,70)
	Emtricitabine	MRP1	P-gp MRP1–3 (≥ 1 μM)	P-gp (2.5x, 1 mM)[c]	(69,70)
	Tenofovir	BCRP MRP2 MRP4	MRP1–3 (≥ 50 μM)		(60,71,72)
	Tenofovir alafenamide	P-gp BCRP			(73)
	Tenofovir disoproxil fumarate	P-gp	P-gp (≥ 500 μM)		(74,75)
	Abacavir	P-gp BCRP MRP4 UGT1A1	P-gp BCRP (385 μM) MRP1–2 (≥ 400 μM)	P-gp (1.5x, 15 μM)[d] CYP2B6 (2.3x, 5 μM)[d]	(57,65,71,74)
Non-nucleoside reverse transcriptase inhibitors	Doravirine	CYP3A4 (20.9 μM)			(76)
	Efavirenz	BCRP CYP2B6 (20 μM) UGT2B7(24 μM)	P-gp BCRP (20.6 μM) MRP1–3 (> 1 μM)	P-gp (2x, 4 μM)[c] CYP3A4 (5.7x, 23 μM)[d] CYP2B6 (4.7x, 10 μM)[d]	(26,63,64,65,70,72,74,77)
	Rilpivirine	CYP3A4 (4.2 μM) CYP2B6	P-gp (4.5 μM) BCRP (1.5 μM) CYP3A4 (1.3 μM) CYP2B6 (4.2 μM) CYP2C19 (2.7 μM)	P-gp (2x, 2 μM)[d] CYP3A4 UGT1A3	(78–81)
Protease inhibitors	Darunavir	P-gp CYP3A4 CYP2D6	P-gp (32.9 μM) CYP3A4	P-gp (1.7x, 10 μM)[d] CYP2B6 (6.1x, 10 μM)[d]	(26,64,65,79)
	Ritonavir	P-gp MRP1–2 CYP3A4 (0.04 μM) CYP2D6 (1 μM)	P-gp (5.4 μM) MRP1 (33.5 μM) BCRP (19.5 μM) CYP3A4 (0.014 μM)	P-gp (2x, 10 μM)[c] MRP2 (5.5x, 10 μM)[d] CYP2C9 (2x, 10 μM)[c] CYP2C19 (2x, 10 μM)[c]	(64,82–87)
	Atazanavir	P-gp MRP1–2 CYP3A4 (1 μM)	P-gp (24.9 μM) MRP1 (42.2 μM) BCRP (69.1 μM) UGT1A1 (2.3 μM)	P-gp (2x, 10 μM)[c]	(25,26,69,74,86,88–90)

[a] ARVs included in the current Department of Health and Human Services guidelines on recommended initial regimen for most people living with HIV, including people in certain clinical situations (4)
[b] f_2 value (concentration needed to increase substrate baseline fluorescence two-fold) reported
[c] Fold-increase in functional activity at the concentration indicated
[d] Fold-increase in mRNA expression, protein expression, or gene promoter activity at the concentration indicated

ARV Efflux Transporters in the Male and Female Genital Tracts

In the male genital tract, our group demonstrated expression and/or function of P-gp, MRP1, and MRP4 *in vitro* at the human and mouse BTB (25). We also observed mRNA and protein expression of P-gp, BCRP, MRP1, MRP2, and MRP4 in testicular tissue of individuals living with HIV undergoing gender reaffirmation surgery (22). Most recently, we demonstrated the expression and/or function of P-gp, BCRP, and MRP1 in CD4+ and CD8+ T cell subsets isolated from uninfected testicular tissue (116). ABC

transporters have also been identified in testicular interstitial cells such as Leydig cells, peritubular myoid cells, and macrophages isolated from mouse, rat, and/or human testes (117). In addition to the testes, mRNA expression of genes encoding P-gp, BCRP, and MRP1-9 was detected in the epididymis, prostate, vas deferens, and seminal vesicles of the rat genital tract (118). Drug efflux transporters and metabolic enzymes present along the male genital tract most likely contribute to low penetration of several ARVs in the seminal fluid, as well as in tissues such as the testis which demonstrate low PI concentrations (22,39).

Studies have examined the expression of drug efflux transporters in human cervicovaginal cell lines and tissues, and their roles in the disposition of ARV-based microbicides in the female genital tract (119,120). Gene expression of P-gp, BCRP, and several MRP isoforms was demonstrated in human and non-human primate cervicovaginal tissues and Ect/E6E7 and End/E6E7 cell lines immortalized from primary ectocervical and endocervical epithelial cells, respectively (119–121). Furthermore, cervicovaginal tissues collected from macaques vaginally treated with film formulations of tenofovir or darunavir displayed significant induction of MRP2 (121). Drug efflux transporters and metabolic enzymes expressed in HIV target cells, as well as along the epithelium, of the female genital tract (Table II) could potentially contribute to low concentrations of ARVs at this site (41,42).

ARV Efflux Transporters in Immune Cells

Multiple cell types in circulating blood can be infected by HIV. The expression and activity of drug efflux transporters and drug metabolic enzymes in PBMCs suggest their contribution to reduced ARV intracellular concentrations and antiviral activities in HIV cell reservoirs. We recently identified P-gp, BCRP, and MRP1 expression and function in CD4+ and CD8+ T cells by performing flow cytometry analyses (116). Furthermore, we assessed their expression in naïve CD4+ T cells compared to central, transitional, and effector memory CD4+ T cells, and demonstrated that MRP1 protein expression was significantly higher in the memory T cell (116). Janneh *et al.* demonstrated increased intracellular concentrations of lopinavir in the presence of ritonavir, atazanavir, and amprenavir, as well as well-established inhibitors of P-gp, MRP1, and MRP2, in vitro in CD4+ T-lymphoblastoid cell lines (98). Minuesa *et al.* reported that HIV infection increased P-gp mRNA expression *in vitro* and further demonstrated a positive correlation between HIV plasma viral load and P-gp activity in primary CD4+ and CD4+CD45RA− memory T cells, as well as increased raltegravir efflux in CD4+ T cells with higher P-gp activity (122). Zhang *et al.* also demonstrated alterations in BCRP expression in CD4+ and CD8+ T cells isolated from people living with HIV, with or without ART, as well as healthy donors. BCRP expression was highest in people living with HIV on ART, and higher in the untreated group, compared to healthy donors (123).

P-gp, BCRP, and MRP1 gene expression has been demonstrated in human peripheral monocytes at low levels (124). Additionally, P-gp has been detected in monocyte-derived macrophages at the protein level, with higher functional activity in macrophages that have been polarized to the M2 phenotype compared to M1 or unstimulated macrophages (63). In a follow-up study from the same group, mRNA and protein expression of BCRP and MRP1 in M1 and M2 monocyte-derived macrophages was demonstrated with M1 macrophages exhibiting higher MRP1 expression and M2 macrophages displaying higher BCRP expression (136). The intracellular concentration of zidovudine and indinavir was shown to be increased in monocyte-derived macrophages following inhibition of P-gp and MRP1 by the inhibitors valspodar and probenecid, respectively, demonstrating functional activities of the transporters in ARV efflux from these cells (137). We recently obtained data demonstrating mRNA and/or protein expression of P-gp, BCRP, MRP1, and MRP4 in monocytes and monocyte-derived macrophages of HIV-negative donors and individuals living with HIV on ART. Furthermore, these transporters demonstrated significantly higher expression in macrophages when compared to monocytes. In addition, ARVs known to be substrates of these transporters and metabolic enzymes were readily detected in monocytes of HIV+ART donors but were lower or undetectable in macrophages (manuscript under review).

The functional expression of drug transporters and metabolic enzymes in dendritic cells is poorly characterized. Laupeze *et al.* investigated the expression and activity of P-gp and MRP1 in human peripheral monocyte-derived dendritic cells. Immunolabeling experiments and dye efflux assays indicated that monocyte-derived dendritic cells displayed elevated levels of MRP1 expression and activity when compared to levels in parental monocytes (127). mRNA expression and/or immunocytochemical analyses of MRP1, MRP3, and MRP5 was also reported in mature monocyte-derived dendritic cells (128), while mRNA and protein expression of BCRP was detected in cultured human monocyte-derived dendritic cells by qPCR and western blot analyses, respectively (125). Moreover, BCRP was demonstrated to be functionally active in these cells after performing mitoxantrone efflux assay (125).

Overall, drug efflux transporters could be differentially expressed and significantly alter ARV concentrations in HIV-infected T cells, monocytes, macrophages, and other cells implicated as HIV reservoirs. The activity of these transporters could result in suboptimal ARV intracellular concentrations which could contribute to the formation of HIV cell reservoirs.

Drug Metabolic Enzymes

Drug metabolism is conventionally divided into Phase I and Phase II processes. Phase I metabolism involves reactions that introduce a polar functional group on a molecule through processes such as oxidation, reduction, or hydrolysis (56). Phase II metabolism involves conjugation reactions, where a highly hydrophilic functional group such as a sulfate or a glucuronide is attached to render the compounds more water-soluble and suitable for elimination (56). The most important enzymes involved in phase I drug metabolism belong to the cytochrome P450 (CYP450) superfamily of enzymes. Isoforms such as CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP2B6 play a key role in metabolizing ARVs (126,129). Key phase II metabolic enzymes include uridine diphosphate-

Table II. mRNA and Protein Expression/Localization of ABC Transporters and Metabolic Enzymes in Sites Recognized as HIV Reservoirs

Drug efflux transporter/ metabolic enzyme	Expression in proposed HIV cellular/anatomic reservoirs		References
	Gene	Protein	
P-gp	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets; monocytes, MDM, CNS [microglia, astrocytes], GI [jejunal mucosal biopsy; recto-sigmoid colon biopsy], MGT [Sertoli cells, Leydig cells, myoid cells, epididymis, prostate, vas deferens, seminal vesicles], FGT [cervicovaginal tissue, ectocervix and endocervix tissues, and epithelial cell lines]	PBMCs, CD4+ T cells, CD8+ T cells, monocytes, MDM, Langerhans dendritic cells, CNS [microglia, astrocytes], GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [Sertoli cells, Leydig cells, myoid cells, testis endothelium], FGT [ovarian epithelium, fallopian tube, endocervical epithelium, endometrial epithelial cells]	(14,108,109,118,119,121–126)
BCRP	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets, MDM, monocytes, MdDC, CNS [microglia, astrocytes], GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [Sertoli cells, whole testis tissue, epididymis, prostate, vas deferens, seminal vesicles], FGT [vaginal, ectocervix and endocervix tissues, and epithelial cell lines]	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets MdDC, Langherans dendritic cells, dermal dendritic cells, MDM, CNS [microglia; astrocytes], GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [myoid cell, testis endothelium, whole testis tissue]	(14,18,115,116,118,119,121,123,124,127–130)
MRP1	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets, monocytes, MDM, MdDC, CNS [astrocytes, microglia], GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [Sertoli cells, whole testis tissue, epididymis, prostate, vas deferens, seminal vesicles], FGT [vaginal, ectocervix and endocervix tissues and epithelial cell lines]	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets; MDM, MdDC, skin dendritic cells, CNS [astrocytes, microglia], GI [recto-sigmoid colon biopsy], MGT [Sertoli cells, Leydig cells prostate gland]	(14,17,108,111,112,118,119,121,123,124,131,132)
MRP2	PBMCs, CD4+ T cell, CD8+ T-cell, GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [epididymis, prostate, vas deferens, seminal vesicles], FGT [vaginal, ectocervix and endocervix epithelial cell lines, ectocervix tissue]	PBMCs, GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [Sertoli cells, Leydig cells, myoid cells]	(118,119,123,124,133)
MRP3	PBMCs, CD4+ T-cells, monocytes, MDM, MdDC, CNS [microglia, astrocytes], GI [jejunal mucosal biopsy], MGT [epididymis, prostate, vas deferens, seminal vesicles], FGT [ectocervix and endocervix tissue and epithelial cell lines, vaginal epithelial cells]	MdDC	(115,117,119,123,124,132)
MRP4	PBMCs, CD4+ T-cells, monocytes, MDM, CNS [microglia, astrocytes], GI [jejunal mucosal biopsy, recto-sigmoid colon biopsy], MGT [Sertoli cells, whole testis tissue, epididymis, prostate, vas deferens, seminal vesicles], FGT [ectocervix and endocervix tissue and cell lines, vaginal epithelial cells]	Skin dendritic cells, CNS [astrocytes], MGT [whole testis tissue]	(113,117–119,123,124,126,132)
MRP5	PBMCs CD4+ T-cells, monocytes, MDM, MdDC, CNS [microglia, astrocytes]; GI [jejunal mucosal biopsy], MGT [epididymis, prostate, vas deferens, seminal vesicles], FGT [vaginal, ectocervix and endocervix tissues and epithelial cell lines]	MdDC, CNS [astrocytes]	(113,117,119,123,124,126,132)
CYP3A4	PBMCs, MDM, monocytes, GI [jejunal mucosal biopsy], MGT [whole testis tissue]	PBMCs, CD4+ T cells, CD8+ T cells, memory T cell subsets, monocytes, MDM, GI [jejunal mucosal biopsy], MGT [whole testis tissue]	(14,119,121,133,134)

Table II. (continued)

Drug efflux transporter/ metabolic enzyme	Expression in proposed HIV cellular/anatomic reservoirs		References
	Gene	Protein	
CYP2D6	Lymphocytes, MDM, GI [jejunal mucosal biopsy], MGT [whole testis tissue]	MDM, CNS [astrocytes], MGT [whole testis tissue]	(14,119,134,61)
CYP2B6	PBMCs, lymphocytes, MDM, monocytes, GI [jejunal mucosal biopsy]	PBMCs, MDM	(119,133,134)
UGT1A1	Rat peripheral lymphocytes, rat peritoneal macrophages, CNS [rat cerebellum], FGT [ectocervix and vaginal tissues]	CD4+ T cells, CD8+ T cells, rat peritoneal macrophages	(121,135,62)
UGT2B7	CNS [cerebellum], GI [jejunal mucosal biopsy]		(119,135)

Expression of transporters and enzymes is reported in human cells/tissues unless indicated otherwise
CNS central nervous system, *GI* gastrointestinal system, *FGT* female genital tract, *MdDC* monocyte-derived dendritic cells, *MDM* monocyte-derived macrophages, *MGT* male genital tract, *PBMCs* peripheral blood mononuclear cells

dependent glucuronosyltransferases (UGT), sulfotransferase, and glutathione-S-transferase (131). Overall, most ARVs are oxidatively metabolized through the CYP450 pathway, while a few are conjugated by the UGT enzymes (129). Interactions of ARVs with these enzyme systems could result in subtherapeutic effects or toxicity (126).

PIs and NNRTIs are commonly implicated in ARV drug interactions since they are primarily metabolized by the CYP450 enzymes. CYP3A4 is involved in the metabolism of all PIs and several NNRTIs, while CYP2D6 is known to metabolize ritonavir, nelfinavir, darunavir, and delavirdine (126). CYP2B6 also plays a role in the metabolism of drugs such as efavirenz and nevirapine (126). Ritonavir, a potent CYP3A4 inhibitor, is associated with the inhibition of CYP3A4-mediated metabolism of other PIs, as such, this drug is used as a pharmacoenhancer of several PIs during ART (132). NNRTIs such as nevirapine and efavirenz are inducers of both CYP3A4 and CYP2B6, while delavirdine can either inhibit or induce CYP3A4, CYP2C9, and CYP2C19 (126). The INSTIs also undergo metabolism by CYP450 and/or UGT phase II metabolic enzymes. Elvitegravir is predominantly metabolized by CYP3A4, with minor contributions from UGT1A1 (130). Dolutegravir and raltegravir are primarily metabolized by UGT1A1; however, dolutegravir also undergoes minor metabolism by CYP3A4 (98,138). Bictegravir (GS-9883) is a novel INSTI that has been shown to be metabolized by both CYP3A4 and UGT1A1 with similar contributions from both metabolic pathways (139). In addition to INSTIs, the NRTI abacavir is a substrate of UGT1A1, while zidovudine and efavirenz are substrates of UGT2B7 (140). Drug metabolism by these enzyme systems could significantly influence the intracellular concentration and efficacy of ARVs at specific sites of infection.

ARV Metabolic Enzymes in HIV Tissue Sanctuaries

The expression and activity of drug metabolic enzymes in the human brain are poorly characterized, possibly due to the large amount of microsomal or mitochondrial protein that is required to purify and quantify these enzymes, as well as their low abundance in the brain tissue. In the human brain, Dutheil *et al.* were able to identify robust expression of CYP2D6 in astrocytes by performing immunostaining experiments (61). Furthermore, a transgenic mouse model expressing human UGT2B7 demonstrated expression and activity of this enzyme in the brain (135). Togna *et al.* also demonstrated UGT1A1 mRNA and protein in primary cultures of neonatal rat microglia in the context of morphine metabolism (141). However, the cellular expression, localization, and function of these metabolic enzymes in the human brain remain to be determined. In the female genital tract, several phase I and II metabolic enzymes were detected in human ectocervix and vaginal tissues, particularly, UGT1A1 displayed high mRNA expression in these tissues (120). In our studies, we demonstrated the expression of CYP3A4, CYP2D6, and UGT1A1 in human testicular tissue, as well as in T cell subsets isolated from testes (22,116). These metabolic enzymes could contribute significantly to the lower ARV concentrations observed in tissue sanctuaries.

ARV Metabolic Enzymes in Immune Cells

Only a few studies have characterized the expression of drug metabolic enzymes in cells that are known targets of HIV. In PBMCs obtained from healthy volunteers, Liptrott *et al.* confirmed the expression of CYP3A4 and CYP2B6 by performing flow cytometric analyses (133). We also demonstrated the expression of CYP3A4 and UGT1A1 in human circulating CD4+ and CD8+ T cell subsets, and further characterized the expression of CYP3A4 in naïve, central, transitional, and effector memory T cell subsets (116). The expression and/or activity of drug metabolic enzymes was also reported in macrophages. Jin *et al.* reported mRNA and protein expression of metabolic enzymes including CYP3A4, CYP2D6, and CYP2B6 in the U937 macrophage cell line. In addition, the functional activity of CYP3A4 was demonstrated in cell extracts using a fluorescent substrate with or without the CYP3A4 inhibitor ketoconazole (134). Most recently, we detected mRNA expression of CYP2B6, CYP2D6, and UGT1A1 in monocytes and monocyte-derived macrophages isolated from the blood of HIV-negative donors (manuscript under review). Overall, studies on the protein expression and functional activity of drug

efflux transporters and metabolic enzymes in monocytes and macrophages, and their contributions to ARV concentrations in these cell types remain limited.

Regulation of Drug Efflux Transporters and Metabolic Enzymes by HIV Infection or ART

ARV-Mediated Regulation of Drug Efflux Transporters and Metabolic Enzymes Via Nuclear Receptors

Nuclear receptors comprise the largest superfamily of gene transcription factors that regulate the expression of their target genes in response to specific ligands (142). In humans, there are forty-eight known nuclear receptor genes which are classified into three groups: endocrine receptors which recognize steroids and hormones, adopted receptors whose endogenous ligands have been identified after their discovery, and orphan receptors with unidentified physiological ligands or activators (143). Several orphan nuclear receptors including the pregnane x receptor (PXR) and the constitutive androstane receptor (CAR) are expressed in many animal species and are recognized as key modulators of drug-induced changes in both metabolism and efflux mechanisms (143). PXR and CAR play overlapping roles in regulating the expression ABC and SLC drug transporters, as well as phase I and II metabolic enzymes, potentially affecting the tissue distribution and elimination of numerous ARVs (144).

Studies have revealed that ARVs can directly activate human PXR and CAR. Using African green monkey kidney epithelial cells (CV-1), it has been shown that PIs such as ritonavir was capable of activating PXR and induced the expression of P-gp, MRP2, and CYP3A4 (64). Our group also demonstrated that efavirenz is a ligand for both PXR and CAR (26,65). We previously screened for interactions between human PXR/CAR and clinically relevant ARVs. Applying luciferase reporter gene assays in HCMEC/D3 cells, we identified that amprenavir, atazanavir, darunavir, efavirenz, ritonavir, and lopinavir are activators of PXR, whereas abacavir, efavirenz, and nevirapine activated CAR (65). Furthermore, treatment with lopinavir, amprenavir, and efavirenz resulted in approximately two-fold induction of P-gp protein expression leading to reduced intracellular concentrations of a fluorescent P-gp probe (i.e., rhodamine-6-G), thereby suggesting increased P-gp function (65). We also demonstrated upregulation of P-gp, Bcrp, and Mrp4 mRNA and protein expression, after exposure to PXR or CAR ligands at the mouse BTB in vitro, and found that exposure to efavirenz and darunavir significantly increased P-gp expression and function *ex vivo* using freshly isolated mouse seminiferous tubules (26). Using the HepG2 cell line to perform luciferase reporter assay, Svard *et al.* demonstrated PXR-mediated induction of CYP3A4 promoter activity in the presence of lopinavir, nelfinavir, fosamprenavir, efavirenz, and tipranavir, while the induction of CYP2B6 promoter activity was shown in the presence of abacavir, efavirenz, darunavir, and lopinavir. In addition, they found CAR-mediated induction of CYP2B6 promoter activity in the presence of lopinavir, fosamprenavir, and tipranavir (145). Together, activation of these xenobiotic nuclear receptors could result in the upregulation of efflux transporters, further limiting ARV intracellular concentrations.

HIV-Mediated Regulation of Drug Efflux Transporters

Several studies by our group and others have investigated the regulation of ABC drug transporters by HIV infection. Particularly, our group demonstrated gp120-mediated downregulation of P-gp in primary cultures of rodent or human fetal astrocytes (111,146). Interactions of HIV-1_{ADA} gp120 with the chemokine co-receptor CCR5 resulted in activation of the transcription factor nuclear factor kappa B (NF-κB) pathway and secretion of several pro-inflammatory cytokines, including IL-6 which had a significant downregulatory effect on P-gp expression and function (111). These results were confirmed using human fetal astrocytes exposed to either R5 tropic (HIV-1_{ADA}) or dual tropic R5/X4 (HIV-1 89.6) viral isolates which resulted in downregulated P-gp protein expression (110). The functional expression of MRP1 was also investigated in primary cultures of rat astrocytes exposed to gp120. The results demonstrated a significant increase in the mRNA and protein expression of MRP1 following exposure to gp120 (112). This increase in MRP1 expression was associated with an enhanced efflux of glutathione and glutathione disulfide (112), which play a key role in oxidative stress. Furthermore, both oxidative stress (112) and the inflammatory cytokine TNFα (147) played a role in mediating the upregulation of MRP1 through the NF-κB and c-Jun N-terminal kinase pathways. Hayashi *et al.* demonstrated that treatment of mouse brain microvascular endothelial cells with HIV Tat protein resulted in increased P-gp mRNA and protein expression and functional activity through NF-κB-mediated mechanisms (109). Furthermore, Tat induced MRP1 expression and function in brain microvascular endothelial cells and astrocytes following activation of the mitogen-activated protein kinase signaling cascade (108).

HIV-1 infection has also been shown to alter the expression of drug transporters in cells and/or tissues obtained from individuals living with the virus. As discussed earlier (section 4.2.2), our group demonstrated that the expression of various drug transporters was altered in upper and lower intestinal tissue biopsies obtained from ART-naïve or ART-treated individuals living with HIV (114,115). In addition, Zhang *et al.* demonstrated increased levels of BCRP expression in CD4+ and CD8+ T cells isolated from individuals living with HIV (both ART-treated and untreated) compared to HIV-negative donors (123). Furthermore, an induction in the expression and function of BCRP was reported in Tat-expressing Jurkat T cell lines compared to control cells (148), implicating a role of this viral protein in inducing BCRP expression. Turriziani *et al.* also demonstrated that the mRNA expression of Pgp, MRP1, MRP4, and MRP5 was significantly higher in PBMCs obtained from people living with HIV who were experiencing virologic failure compared to control HIV-negative donors (149). An early study by Gupta and Gollapudi demonstrated induced expression and activity of P-gp in the efflux of zidovudine and daunorubicin in the HIV-infected H9 T cell line and the U937 monocytic cell line (66). Lastly, Minuesa *et al.* reported that HIV-1 infection increased P-gp mRNA expression *in vitro* and further demonstrated a positive correlation between HIV-1 plasma viral load and P-gp function in primary CD4+ and CD4+CD45RA− memory T cells (122). These studies suggest that the functional expression of drug efflux

transporters could be increased and potentially alter ARV concentrations in HIV-infected target cells.

CONCLUSION

It is now well recognized that drug transporters and metabolic enzymes contribute to drug absorption, distribution, metabolism, and elimination, potentially leading to clinically significant alterations of drug pharmacokinetic and pharmacodynamic properties. Members of the ABC family of drug transporters are known to be expressed at key tissue barriers such as the BBB and the BTB, in epithelial and/or endothelial cells of the female genital tract and the gastrointestinal tract, as well as in lymphocytes and myeloid cells, and are believed to play an important role in regulating drug pharmacokinetic properties, particularly the distribution of ARVs into tissues. It remains unknown whether differential expression and activity of drug efflux transporters and/or metabolic enzymes in various immune cell types could influence their abilities to harbor and maintain latent HIV infection. Further work is needed to investigate drug transporters and metabolic enzymes and their contributions to suboptimal ARV antiviral activities in HIV target cells, particularly those maintaining the tissue reservoirs. In addition, the mechanisms regulating the expression and activities of these transporters and enzymes following HIV infection should be elucidated. A thorough understanding of the functional expression of drug transporters and metabolic enzymes in cell types and tissues that are characterized as HIV reservoirs and sanctuaries could provide insights on antiretroviral drug penetration and antiviral response in these sites. More efforts should be directed at developing alternative approaches to achieve adequate intracellular ARV concentrations and enhance drug penetration, while minimizing drug toxicity in HIV reservoirs and sanctuary sites. Furthermore, therapeutic drug monitoring and the development of individualized therapy could improve patient-specific treatment efficacy and safety of ARVs.

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Contribution by Authors as Women Scientists

Sana-Kay Whyte-Allman

Sana-Kay Whyte-Allman is a PhD student at the Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, where she is supervised by Dr. Reina Bendayan. Sana-Kay conducts research to investigate the role and regulation of membrane-associated drug transport proteins and drug metabolic enzymes in HIV antiretroviral drug disposition, and their potential contribution to persistent HIV infection. Her work has resulted in several first authorship and co-authored publications, international and national conference presentations, and travel scholarships. Sana-Kay is also a recipient of the prestigious University of Toronto Connaught International Doctoral Scholarship for the duration of her PhD studies. As an international student from Jamaica, Sana-Kay represents a first-generation college/university student from a minority group, and an accomplished young researcher with a strong desire to make significant contributions to the pharmaceutical science field.

Reina Bendayan

Dr. Reina Bendayan is a professor of the Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, and career scientist of the Ontario HIV Treatment Network, Ministry of Health of Ontario. Dr. Bendayan's research program is primarily focused on Membrane Transport and Therapeutics with an emphasis in the field of HIV/AIDS Antiviral Drug Transport and Regulation at sanctuary sites and cellular reservoirs of HIV. Her research program is primarily funded by the Canadian Institutes of Health Research, Ministry of Health of Ontario, and the Natural Sciences and Engineering Research Council of Canada. She is the author of over 100 peer-reviewed manuscripts, and over the course of her academic career, she has supervised many undergraduate research students, master's students, doctoral students, and post-doctoral research fellows. Dr. Bendayan understands the immense value of welcoming diversity into her laboratory and over the past years has put significant efforts in ensuring an inclusive research environment in her laboratory including men, women, and individuals from under-represented groups. Dr. Bendayan was elected Fellow of the AAPS (2010) and the Canadian Society of Pharmaceutical Sciences (CSPS, 2015), and received the Association of Faculties of Pharmacy of Canada Research Career Award (2013) and the CSPS Research Leadership Award (2019). She served as Graduate Chair and Associate Dean Graduate Education of the Graduate Department of Pharmaceutical Sciences (July 2005–July 2011) and as Acting Dean of the Leslie Dan Faculty of Pharmacy (January 2007–July 2007).

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