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Overcoming pharmacologic sanctuaries

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Abstract

Purpose of review—Current antiretroviral treatment regimens represent significant improvements in the management of HIV-1 infection; however, these regimens have not achieved a functional or sterilizing cure. One barrier to achieving a cure may be suboptimal antiretroviral concentrations in sanctuary sites throughout the body, including the central nervous system, gut-associated lymphoid tissue, lymph nodes, and tissue macrophages. This review will focus on the problems associated with achieving effective concentrations in these restricted sanctuary sites, and potential strategies to overcome these barriers.

Recent findings—Sufficient data exist to conclude that antiretroviral drug distribution is not uniform throughout the body. Low tissue/reservoir concentrations may be associated with viral replication. Multiple means to increase drug concentrations in sanctuary sites are being investigated, including modification of currently utilized drugs, blockade of transporters and enzymes that affect drug metabolism and pharmacokinetics, and local drug administration. Accumulating data suggest these methods increase antiretroviral concentrations in reservoirs of viral replication. No method has yet resulted in the complete clearance of HIV.

Summary—New strategies for increasing antiretroviral concentrations in predominant sites of viral replication may provide more effective means for elimination of viral sanctuaries. Additional research is necessary to optimize antiretroviral tissue distribution in order to inhibit virus replication fully, and avoid resistance and replenishment of viral reservoirs that may persist in the face of antiretroviral therapy.

Keywords

HIV-1; resistance; sanctuary; transporter

INTRODUCTION

The use of combination highly active antiretroviral therapy (ART) can achieve levels of HIV-1-RNA in plasma (viral load) that are undetectable by conventional assays in the vast majority of HIV-1-infected individuals [1–3]. In spite of this factor, there is growing evidence for residual viral replication in patients on suppressive ART [4[■],5]. There is also evidence for low-level viremia in ART-suppressed patients, although the origin of this low-

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Conflicts of interest

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level viremia and whether it is a result of residual viral replication is unclear [6]. Neither a sterilizing cure, defined as a complete elimination of HIV-1 from the body, nor a functional cure, in which there is no viral replication, but all traces of the virus have not been eliminated, has been achieved through ART [7]. Upon discontinuation of ART, there is a rapid recrudescence of viremia from long-lived reservoirs, leading to a decline in clinical parameters and the development of resistance [8,9,10[■]]. There may be multiple obstacles to achieving complete clearance of the viral reservoirs, but one hypothesis, drawn from data demonstrating pharmacologic sanctuaries for antiretroviral (ARV) drugs in animals, is that concentrations of all agents in the ART regimens are not sufficient to fully suppress viral replication in all reservoir compartments. In this review we will discuss the locations and properties of these reservoirs, as well as potential strategies to achieve clinically effective concentrations in these reservoirs.

PHARMACOLOGIC SANCTUARIES

In general, drugs do not distribute evenly throughout the whole body [11[■],12,13]. The reasons for this are complex and multifactorial. Absorption and distribution vary by drug and by location depending on the physicochemical properties of the drug, the size and rate of perfusion of the target site, and protein-binding of the drug. Additional factors include if a drug is a substrate of influx or efflux transporters, metabolic enzymes, and patient-specific polymorphisms for transporters and metabolic enzymes. These complex factors can lead to significant differences in drug concentrations in different compartments, as well as different drug concentrations in these compartments between patients. Whereas measurements of drug concentrations in plasma are easy to obtain, the concentrations in restricted compartments and the sites of action for a given drug are considerably more complex to obtain and to quantify. One illustrative study utilized [¹⁸F]FMPA, a radiolabeled tenofovir analog, to determine distribution in rats. [¹⁸F]FMPA concentrations in spleens and mesenteric lymph nodes were two-fold lower than plasma concentrations, mesenteric lymph nodes and testes were four-fold lower, and brain concentrations were 25-fold lower than plasma concentrations [14]. Differences in drug concentrations in peripheral compartments may limit the inability of ART to fully suppress HIV-1 replication. In HIV-1-infected patients, Solas *et al.* [15] assessed concentrations of indinavir, nelfinavir, and lopinavir in plasma and a number of sanctuaries including cerebrospinal fluid (CSF), lymphoid tissue, and semen, and found considerable variation in drug concentrations between these sites, and between the various medications.

Gut- and rectal-associated lymphoid tissue

Lymphoid tissue in the gut (gut-associated lymphoid tissue, or GALT), as well as in the rectum (rectal-associated lymphoid tissue, or RALT) is the major site of viral replication in nonvirologically suppressed patients and may also serve as a sanctuary for HIV-1. Even in the presence of long-term ART, there are reports of HIV-1 residing in this tissue [16]. Chun *et al.* [16] have suggested that latently infected T cells in the gut comprise a key reservoir in HIV-1-infected patients. They observed significantly higher levels of HIV-1 DNA normalized to CD4⁺ T-cell count in GALT tissue than in either resting or activated CD4⁺ T cells in the blood [16]. They found high degrees of sequence similarity between virus in the GALT and virus in peripheral blood mononuclear cells (PBMCs), suggesting the gut provides a site for further infection of circulating PBMCs. Others have shown similarity between HIV-1 sequences in the gut and plasma in HIV-1-positive individuals, suggesting that even with undetectable HIV-1 RNA in plasma, these patients still have ongoing replication in the gut, and that there is equilibrium between these two compartments [17[■], 18]. Additional studies suggest viral concentrations are higher along the entire gastrointestinal tract as compared with the blood of HIV-1+ patients treated with ART [19]. Ratios of unspliced RNA to DNA were also lower in the colon and rectum of these patients,

compared with PBMCs, suggesting a low rate of HIV-1 transcription [19]. Latent virus in this reservoir can be released into the general circulation [4[■]].

The causes for these differences in viral burden are likely multifactorial. One pharmacologic explanation is the expression of drug-metabolizing enzymes and transporters is not uniform along the gastrointestinal tract; this would be expected to give rise to regional differences in antiretroviral concentrations. For example, considerable differences in expression for transporters including BCRP, multi-drug resistant protein (MRP) 2, and P-glycoprotein (P-gp) exist among patients and different sections of the gut [20,21[■]]. Small differences in P-gp, BCRP, OCTN2, and MCT1 expression occur along the length of the small intestine [20]. The colon has higher expression of MCT1, OCTN2, and MRP3 than the small intestine [20]. Human studies have shown considerable variation in cytochrome P450 (CYP) enzymes, including CYP3A expression along the length of the gastrointestinal tract, with the majority of CYP expression occurring in the proximal section of the gut [22,23]. These differences may contribute to the presence of sanctuary sites that afford the virus some degree of protection from antiretroviral suppression.

Lymph node

Mesenteric lymph nodes have been identified as a sanctuary site. Horiike *et al.* [10[■]] found effective suppression of plasma viral RNA in simian immunodeficiency virus (SIV)-infected macaques treated with ART. However, when they examined tissue from these animals, they found detectable virus levels in the spleens and lymph nodes [10[■]]. Detectable vDNA and vRNA in lymphoid tissue collected at necropsy has been shown in aviremic ART-treated macaques infected with a chimera of SIV containing HIV-1 reverse transcriptase [24]. These animals were treated with efavirenz, emtricitabine, and tenofovir for 26 weeks. Detectable virus was found in a variety of lymph nodes, including mesenteric, axillary, inguinal, iliac, and cervical lymph nodes [24]. Bourry *et al.* [25] observed that in SIV-infected macaques treated with zidovudine/lamivudine/indinavir (ZDV/3TC/IDV), there were low concentrations of 3TC in lymphoid tissue. 3TC concentrations in these compartments were inversely correlated with virus persistence in these compartments [25]. Solas *et al.* [15] reported IDV concentrations in lymph nodes approximately two-fold greater than in plasma, whereas nelfinavir displayed a ratio of 0.58, lopinavir 0.21, and ritonavir 0.64 of drug concentration in the lymph node to drug concentration in plasma. The inability of the body to clear virus from this compartment in the presence of ART supports a hypothesis of suboptimal drug distribution and underscores one challenge to achieve complete antiretroviral suppression and eradication of the virus.

Central nervous system and genital tract

The central nervous system (CNS) and both the male and female reproductive tract are sanctuaries for HIV-1 [26,27,28[■],29–33]. ARV distribution to these compartments has been extensively discussed and this review will not focus on these sites.

Macrophages

Macrophages, in general, and intestinal macrophages, in particular, are thought to be a reservoir for HIV-1. Zalar *et al.* [18] assessed the presence of virus in duodenal tissue. Similar to the findings of others, they found detectable virus despite undetectable plasma viremia. They performed flow cytometry and PCR on duodenal macrophages, and found that 0.06% of the lamina propria mononuclear cells were HIV-1-infected [18]. Whereas this percentage appeared low, given the large number of cells in this location, this actually represented a significant number of infected cells. Infected macrophages may function as a 'Trojan horse' for the spread of virus throughout the body, most notably to the CNS [34]. Monocytes and macrophages express efflux transporters, including P-gp and MRP, which

may result in decreased intracellular concentrations of antiretrovirals medications that are substrates [35–37]. Jorajuria *et al.* [37] investigated the antiretroviral properties of ZDV and IDV in human monocyte-derived macrophages. They observed greater inhibition of HIV-1 replication by ZDV and IDV in cells also treated with P-gp or MRP1 inhibitors [37]. This indicates that efflux transporters expressed on macrophages maintain sub-therapeutic concentrations in these cells, and explains a potential mechanism by which macrophages may function as a sanctuary for HIV-1. It is worth noting that differences exist in the intracellular environment between macrophages and T lymphocytes, which may result in some antiretrovirals working either more or less effectively in these cells [38].

OVERCOMING PHARMACOLOGIC SANCTUARIES

In patients on suppressive ART, the persistence of virus in sites such as the GALT, RALT, and lymph node may contribute to ongoing immune activation, incomplete immune reconstitution, abnormal vaccine responses, and reduced life expectancy compared with age-matched controls. This, coupled with evidence for poorer ARV distribution to these tissue sites, illustrates barriers to achieving either a functional or sterilizing cure for HIV-1 infection. Overcoming these pharmacologic sanctuaries with new drugs or new drug delivery approaches that achieve drug concentrations in lymphoid tissues that fully suppress virus replication should prevent maintenance of persistent virus reservoirs, and thereby improve prospects for full immune reconstitution and a functional or sterilizing cure of HIV-1 infection.

Compartment-targeted therapy

Whereas few treatments for HIV-1 have used the strategy of targeting specific compartments of ARVs for eliminating virus in these sanctuaries, this approach is being utilized for other diseases, and may hold promise for eliminating HIV-1 from restricted sanctuaries. The treatment of individuals with childhood acute lymphoblastic leukemia commonly includes intrathecal chemotherapy, with minimal long-term side effects [39]. Similarly, patients with cytomegalovirus retinitis have been treated with intravitreal ganciclovir [40]. In the treatment of HIV-1, local administration is primarily being investigated for microbicides, but may theoretically be of use for residual virus replication in sanctuary compartments. Studies assessing microbicides in primates treated with either intra-vaginal or intrarectal administration of tenofovir have displayed detectable concentrations for 24 h postadministration of the drug product [41]. Similar studies utilizing a 1% tenofovir gel vaginally in monkeys found concentrations in vaginal lymphocytes greater than the IC95 for the virus at 4 and 24 h postadministration [42].

Transporter and metabolism blockage

Strategies to increase concentrations in sanctuary sites include use of drugs to block influx and efflux transporters or the metabolism of ARVs. Ritonavir, and now cobicistat, have established roles in HIV pharmacotherapy as CYP3A enzyme inhibitors [43[■],44]. In-vitro research suggests that ritonavir and cobicistat also enhance intestinal absorption. Studies with Caco-2 monolayers (a cell line derived from human epithelial colorectal adenocarcinoma cells, commonly used in in-vitro absorption studies) show increased transport of ARVs including atazanavir, darunavir, and tenofovir alafenamide fumarate (GS-7340) across the monolayers [45[■]]. This result is accomplished in part by inhibition of P-gp and BCRP. Similar findings have been observed for ritonavir [46[■]]. This inhibition of efflux transporters in the gut may lead to increased concentrations in plasma, but it is not known if this increase will have a direct effect on drug concentrations in compartments. Namanja *et al.* [47[■]] have recently reported an abacavir prodrug dimer that can inhibit P-gp efflux. The prodrug was designed to increase abacavir CNS penetration. They utilized in-

vitro assays, as well as a brain capillary model, and found this molecule was able to inhibit P-gp in the cell culture system, and is converted back to abacavir inside cells [47[■]]. They suggest that this approach may be utilized for other antiretrovirals as a means to increase penetration past the blood–brain barrier. P-gp blockers have been studied as adjunctive treatment in cancer, but no therapeutic benefit has been established [48].

Nanomedicine

Nano-scale antiretroviral therapies (nanoART), usually defined as ARVs formulated into particles with a size of less than 100 nm, have been proposed as an approach to increase concentrations of ARVs in sanctuary sites [49[■],50,51]. Human monocyte-derived macrophages (MDMs) exposed to nanoART consisting of atazanavir, efavirenz, and ritonavir show rapid uptake of the nanoART, and slow release over an extended time period (15–20 days) [52]. These nanoART products can be utilized to establish drug depots for a sustained response, allowing weekly, and even less frequent administration [49[■]]. Significant concentrations of nanoART products are found in a variety of tissues, including the liver, spleen, kidneys, and lungs of animals for a number of days postadministration [49[■]]. As circulating monocytes and macrophages distribute throughout the body, including to the brain and secondary lymphoid tissue, this factor has been proposed as a means to overcome barriers preventing access of antiretrovirals [52,53]. Nanoformulated ART engulfed by mononuclear phagocytes cocultured with human brain microvascular endothelial cells (HBMECs) can effectively transfer engulfed ART to these cells [54[■]]. When the nanoART was coated with folate they found even higher uptake in the HBMECs; folate-coated nanoART given to mice achieved three-to four-fold greater concentrations in the brain than noncoated[54[■]]. Kinman *et al.* [55] developed a lipid-IDV nanoparticle that resulted in a six-fold increase in IDV lymph node concentrations in macaques treated with the nanoparticle product as compared with a soluble formulation of IDV. Endsley and Ho [56[■]] have recently described peptide-coated lipid nanoparticles of IDV as a means to target the nanoparticles to CD4+ cells, and observed significantly higher antiviral activity. The development of nanoproducts may play an important role in overcoming barriers to achieving adequate concentrations in sanctuary compartments and further study is warranted [57].

Modification of drugs and prodrugs and new drug development

The process of modifying the chemical structure of existing ARVs and developing new ARVs with improved drug distribution properties offers another approach to improve pharmacokinetic/pharmacodynamic properties in sanctuary sites. Tenofovir alafenamide fumarate (GS-7340) is a prodrug of tenofovir with improved penetration into lymphoid tissue in clinical development. [58]. The drug displays variable absorption, with larger doses resulting in a greater percentage of the dose being absorbed than with lower doses, and is very stable in intestinal tissue [59[■]]. This situation is believed to be due to the ability of the drug to saturate intestinal transporters, including P-gp [59[■]]. After oral administration, GS-7340 achieves concentrations in lymph nodes 5–15-fold higher than after oral administration of tenofovir disoproxil fumarate (TDF) [60]. GS-7340 produces intracellular concentrations in PBMCs approximately 150-fold higher than in plasma. This high degree of intracellular penetration results in GS-7340 having a selectivity index (a measure of the cytotoxic concentration to the effective concentration) approximately 10-fold higher than that of TDF [60]. Another example of drug formulation technology to enhance compartment penetration is a conjugate of zidovudine and ursodeoxycholic acid [61[■]]. Ursodeoxycholic acid is capable of penetrating the blood brain barrier, and it is proposed this conjugate may allow increased concentration in the CNS. In-vitro studies utilizing human retinal pigment epithelium layers show higher permeation from the apical to basolateral compartments of

the cells, as compared with the opposite, showing increased permeability of this drug product [61].

CONCLUSION

Accumulating data support the presence of pharmacologic sanctuaries and the possibility that they are an impediment to achieving a functional or sterilizing cure for HIV-1 infection with current ART. In sanctuaries such as the lymphoid tissue, antiretroviral concentrations appear to be insufficient to achieve full suppression of virus replication, and these sanctuaries appear capable of providing a site for development of resistance. Based on the fact that lymphoid tissues are the primary site of HIV-1 replication, the presence of these pharmacologic sanctuaries point to a fundamental gap in our understanding of ARV pharmacokinetic and pharmacodynamic characteristics necessary to achieve complete suppression of HIV-1 replication at its source. A number of strategies are available to improve drug concentration in these sanctuaries, either by increasing plasma concentrations, increasing drug penetration, or enhancing drug delivery. Additional research and development, and the translation of promising approaches to overcome pharmacologic sanctuaries into clinical investigations, are fundamental steps to a cure for HIV infection.

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KEY POINTS

- Current antiretroviral therapies are unable to achieve complete suppression of viral replication throughout the body.
- Residual viral replication and the development of resistance may arise in part because the concentrations of current antiretrovirals (ARVs) in sanctuary sites, such as lymphoid tissue, are suboptimal.
- Potential strategies to improve antiretroviral penetration into sanctuaries include targeting sanctuary sites specifically, blockade of drug metabolism or influx/efflux transporters, modification of drug products to increase absorption and distribution, and nanomedicine.