The mechanisms that control intracellular penetration of the HIV protease inhibitors

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Background

Human immunodeficiency virus (HIV) replicates within cells, such as the T4 lymphocyte. Current drug treatment for HIV infection interrupts the viral lifecycle, at the HIV reverse transcriptase and HIV protease steps (Figure 1). Consequently, antiretroviral drugs must enter cells to inhibit viral replication. This article focuses on the penetration of the HIV protease inhibitors (PIs) and some possible mechanisms that control this process.

Plasma drug concentrations

The oral bioavailability and systemic clearance of a drug are the important determinants of plasma concentrations. Following oral administration, the PIs are primarily metabolized by cytochrome P450 (CYP) 3A4, although CYP 2D6 and CYP 2C9 are also involved.1,2 The PIs are also substrates for the transmembrane efflux pump P-glycoprotein (P-gp) in epithelial cells. Thus, extensive first pass metabolism contributes to low and variable bioavailability. To help ensure that adequate drug concentrations are achieved throughout the dosing interval, therapeutic drug monitoring of the PIs in plasma is increasingly used.

Protein binding

The total plasma concentration is a composite of both unbound and bound drug. Consequently, binding to plasma protein reduces the fraction of drug available for penetration into infected cells and tissues. Most PIs are highly bound to plasma proteins, nelfinavir (99% bound), lopinavir (99%), saquinavir (98%), ritonavir (98%), amprenavir (90%) and indinavir (60%).3,4 Ideally, determination of both total and unbound plasma drug concentrations is necessary to gain an understanding of drug available for pharmacological (virological) effect. Furthermore, fluctuations in the fraction of unbound drug may impact on drug penetrating to the active site. PIs are primarily bound to α1-acid glycoprotein, which has a number of genetic variants and shows variable expression in HIV patients.4 Increasing the amount of extracellular α1-acid glycoprotein alters the intracellular concentration of the PIs in vitro, suggesting that changes in α1-acid glycoprotein may alter in vivo drug penetration into cells.5

Intracellular PI concentrations

In HIV pharmacotherapy, it is important to consider intracellular drug concentrations at the level of the lymphocyte. The distribution of the PIs from plasma into cells and tissues is dependent on many factors, including uptake mechanisms and the relative affinities for cells and tissues versus plasma components. The drugs show differential accumulations within lymphoblastoid cell lines6 and peripheral blood mononuclear cells of virologically suppressed patients in vivo, with nelfinavir > saquinavir > lopinavir > ritonavir > indinavir7 (Figure 2). The importance of measurement of intracellular concentrations is illustrated in this study; patients who were receiving saquinavir hard-gel as sole PI had higher intracellular/plasma ratios than those on saquinavir soft-gel. As virologically suppressed patients receiving saquinavir hard-gel (which has very low bioavailability), these represent a highly selected group of patients. However, the fact remains that these individuals achieved virological suppression despite low plasma saquinavir concentrations. Their adequate antiviral response may be accounted for by the increased drug accumulation.

The degree to which each PI is associated with the cell is a dynamic balance between influx, efflux and sequestration.

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In another study, the co-administration of lopinavir and ritonavir resulted in a greater accumulation of ritonavir in cells compared with other PI regimens containing ritonavir, suggesting that some of the processes that govern intracellular accumulation may be amenable to therapeutic manipulation.

Mechanisms of intracellular accumulation

The mechanism of intracellular accumulation of the PIs remains unknown. Is the drug unbound, embedded in lipid bilayers or complexed to proteins? The distribution of a PI into the cell will depend on its physiochemical properties such as dissociation constant ($pK_a$; the pH at which a drug exists in equimolar amounts of un-ionized and ionized form) and partition coefficient (a measure of the un-ionized compound’s ability to passively diffuse into cells). A number of other factors may be responsible for intracellular penetration, including active transport into cells and sequestration, due to either intracellular protein binding or ion trapping.

Lipophilicity

In order to penetrate the cell, the PIs must either passively diffuse through the lipid membrane or enter by active transport. The partition coefficient is a measure of lipophilicity, the more lipid soluble the PI, the greater its ability to cross the cell or cellular compartment barrier. All the PIs, except indinavir, are highly lipophilic and easily traverse cellular membranes.

Intracellular protein binding

In the plasma, the PIs are highly bound to proteins such as $\alpha_1$-acid glycoprotein; however, within the cell there are also many protein-rich areas (e.g. microfilaments, microtubules, proteins embedded in cellular, nuclear and mitochondrial membranes, and phospholipids and proteins in the endoplasmic reticulum). The PIs are highly bound to extracellular proteins, it seems feasible that the PIs may also be highly bound to intracellular proteins. However, ritonavir, saquinavir, nelfinavir and lopinavir show similar plasma protein binding, yet their hierarchy of cellular accumulation is different. These data imply that protein-mediated sequestration is not solely responsible for the intracellular localization of the PIs.

Ion trapping

As weak bases, the PIs are more likely to cross the lipid membranes of cells and organelles when the pH is greater than the $pK_a$ of the PIs. Secondly, there is a potential for ion trapping in acidic compartments, resulting in accumulation.

Drug transporters

Although penetration and sequestration of drug will impact upon the amount of drug associated with the cellular compartment, the dynamic equilibrium between cell and plasma will also be influenced by drug efflux mechanisms. This involves transport across one or more biological barriers, which may be influenced by a number of factors. It is now recognized that processes such as membrane-bound transport systems are important. A number of transport proteins have been identified by biochemical and molecular cloning methodologies in organs of importance in drug disposition, such as the intestine, liver and kidney (Table 1).

The proteins that have been most extensively studied in the context of lymphocyte efflux of PIs are P-gp and multi-drug resistance-associated protein 1 (MRP1).
P-glycoprotein

P-gp is a 170 kDa membrane glycoprotein. Two closely related genes in humans, *ABCB1* (also known as *MDR1*) and *ABCB4* (also known as *MDR2* or *MDR3*), encode P-gps. The *ABCB1* gene product codes for the transporter implicated in efflux of hydrophobic (cationic) compounds, whereas the *ABCB4* gene product mediates the canalicular secretion of phosphatidylcholine into bile. However, evidence has suggested a role for *ABCB4* P-gp in the transport of a certain subset of cytotoxic P-gp substrates.

**P-gp distribution**

P-gp has been observed in liver, colon, jejunum, kidney, pancreatic ductules and adrenal. More recently, P-gp expression has been observed in components of the blood–brain and blood–testis barriers and this may have implications for limiting PI penetration to these organs, with the resultant formation of sanctuary sites for the virus. Normal human lymphocytes express P-gp; we have recently confirmed that different cell subsets express variable levels of the protein. CD56+ cells have the highest level of expression followed by CD8+ then CD4+ lymphocytes.

**Impact of P-gp on intracellular accumulation**

P-gp expression in the lymphocyte alters the in vivo accumulation of PIs, raising the possibility that variation in P-gp expression may reflect treatment outcome on highly active antiretroviral therapy. These issues have fuelled the investigation of the role of genetic variations in the *ABCB1* gene on P-gp, drug exposure and immune recovery in HIV and a correlation between the C3435T single nucleotide polymorphism (SNP) was recently observed. The C3435T SNP is a non-coding, non-promoter SNP at a wobble position in exon 26. It is thus unlikely to affect *ABCB1* gene expression per se, but may be linked to functionally important SNPs in the promoter or enhancer regions of the *ABCB1* gene, or in sequences important for messenger RNA processing. Interestingly, this SNP has also been related to the extent of induction (in response to P-gp inducers) of P-gp, and recent studies in our laboratory have indicated that some PIs induce this protein in vitro. Further investigations are now required to determine whether PIs cause a reduction in their own accumulation via this mechanism.

**Relationship between P-gp and CYP 3A4**

When considering drug accumulation in the lymphocyte it is also important to remember that the PIs are metabolized by CYP 3A4. CYP 3A4 is expressed in lymphocytes and a synergic effect between P-gp and CYP 3A4 has been reported for indinavir in the intestine. This raises the possibility that CYP 3A4 may be an important consideration for PI lymphocytic accumulation. It is interesting to note that the C3435T polymorphism in *ABCB1* has been reported to impact on CYP 3A4 expression in intestine. Clearly, these investigations must now be extended in order to assess interplay at the level of the lymphocyte.

**MRP1**

Given that the PIs have also been shown to be substrates for MRP1, it would seem fair to expect that the arguments presented above hold true for MRP1. The *ABCC1* gene encodes MRP1, and this 190 kDa protein shares 15% sequence homology with P-gp. Furthermore, there is large overlap in both tissue distribution and substrate recognition between the two proteins, raising the possibility of interplay between the two transporters. To date, there have been no functional polymorphisms identified in this protein. A recent study has indicated that PIs may also be substrates for MRP2, but this protein is not highly expressed in lymphocytes and thus is unlikely to impact on PI accumulation.
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**Distribution within the cell**

Subcellular fractionation (into mitochondrial, nuclear, cytosolic and endoplasmic reticulum compartments) illustrates that PIs are distributed in all fractions of the cell. This suggests that the cell-associated drug is within the cellular compartment. After correcting for the amount of protein in each fraction, no pattern was demonstrated, also suggesting that the accumulation is not a result of differing protein content. It should also be remembered that P-gp is present at intracellular locations, raising the possibility of intracellular micro-sanctuary sites.

**Summary**

The PIs are a class of drugs whose members show different physiochemical properties. Because of these properties, the PIs have differential accumulations within cells. As the concentration of free drug at its site of action determines its activity, any mechanism that alters this concentration will alter response. It is therefore essential that these mechanisms are investigated further.

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**References**


