Clinical Pharmacology of Protease Inhibitors In HIV Infection

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**Introduction**

The past few years have reformed the manner in which management and monitoring of HIV patients has taken place. Improved therapeutic regimes and developments have allowed the movement away from monotherapy, to that of combination therapy. Protease Inhibitors are the recently developed drugs (1995) that are now used in triple drug therapy to lower viral load, increase CD4+ T counts, and improve clinical prognosis. Sensitive laboratory methods have been devised, which allow for the quantification of these parameters thereby assisting in the proper care of patients. This paper will deal specifically with the characteristics of protease inhibitors in the clinic, and their appropriate administration, as well as examining their monitoring and consequences of their pharmacokinetic attributes.

**HIV-1 Pathogenesis**

The Human Immunodeficiency Virus 1 (HIV-1) is characterized by the infection and destruction of CD4+ helper T Lymphocytes. This retrovirus consists of single stranded RNA whose viral envelope protein, gp120, adsorbs to the surface of both CD4+ cells and monocytes. Viral entry and uncoating subsequently occurs whereby the enzyme, Reverse Transcriptase (RT), duplicates the single-stranded RNA, into double stranded DNA. Viral integration into the host DNA proceeds, causing the transcription and translation of HIV-1 genes. Chronic infection and ultimate cellular destruction will ensue in these cells, contributing to the progressive destruction of CD4+ cells. The viral proteins and genomic RNA are then assembled into a newly formed viral particle, which by the process of viral budding, dissociates itself from the host cell. The gag and pol genes encode for the viral capsid proteins and for RT, and are expressed as a large precursor protein. The enzyme, HIV-1 Protease cleaves this polypeptide which allows for viral infectivity and replication to transpire. This post-translational modification is essential for the propagation of the virus, as a non-functional HIV-1 protease enzyme will not proteolytically cleave this polypeptide, thereby preventing further development and maturation of these nascent viral particles into infectious virions.

The ultimate goal in the treatment of HIV infection is to reduce the viral load, and to postpone the emergence of opportunistic infections, inevitably prolonging life. In the initial stages, the virus continuously replicates at sustained and high levels. There is no viral latency period, although there may be a period of clinical latency. The plasma life span of HIV is very short. This is suggestive of a very high rate of viral replication. Unfortunately, the RT enzyme has a large degree of error, hence promoting the formation of different HIV variants. This is a major problem, as resistance is most likely to develop, and therapy may be compromised as the virus may become resistant to therapeutic agents. Although the patient’s immune system mounts a strong response, the viral load will ultimately promote the infection and destruction of an increasing number of CD4+ cells, leading to the emergence of opportunistic infections.

Improved laboratory techniques have allowed for the accurate measurement of HIV viral RNA by polymerase chain reaction. Detectable levels of less than 20 copies/mL have been reported. These developments have assisted in the strategy for initiating antiretroviral therapy. As the viral burden becomes established, each patient has a predetermined set point for viral infection. This marker of disease progression is an extremely strong indicator

<table>
<thead>
<tr>
<th>Table 1: Antiretroviral drugs used in HIV Infection</th>
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<tbody>
<tr>
<td>Nucleoside Reverse Transcriptase Inhibitors (NRTI)</td>
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<tr>
<td>Zidovudine (AZT)</td>
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<tr>
<td>Didanosine (ddI)</td>
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<tr>
<td>Zalcitabine (ddC)</td>
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<tr>
<td>Lamivudine (3TC)</td>
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<tr>
<td>Stavudine (d4T)</td>
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</tbody>
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of the patient’s prognosis. A low viral set-point, such as 10,000 copies/ml, is indicative of a slow rate of disease progression, whereas patients with higher levels, 10^5 copies/ml, may have an onset of AIDS in a shorter time period. HIV RNA levels may then predict clinical benefit. CD4+ counts assist the caregiver in predicting disease progression and in initiating and implementing the treatment for the treatment of opportunistic infections. Therefore, the goal of antiretroviral therapy is primarily to reduce the plasma HIV RNA levels, and to magnify the CD4+ T cell levels.

**REVERSE TRANSCRIPTASE INHIBITORS**

There are three groups of antiretroviral drugs that are commonly used. They include the nucleoside reverse transcriptase inhibitors (NRTI), the non-nucleoside reverse transcriptase inhibitors (NNRTI), and the protease inhibitors (PIs), as seen in Table 1. The NRTI and NNRTI groups inhibit RT, preventing the formation of newly formed virions by infected cells. These drugs will somewhat retard disease progression by increasing the CD4+ levels. However, monotherapy has proven to have short lasting effects as resistant HIV mutants have appeared. Combining two nucleosides may prove to have superior effects in patients, albeit limited as well. Another major drawback is that of toxicity. The NRTIs are quite toxic, and may, if co-administered with other drugs, promote haematological or other abnormalities.

**PROTEASE INHIBITORS**

The introduction of PIs has revolutionized the treatment of HIV patients. These drugs cause the infected cell to release immature and non-infectious particles. Unlike the RT inhibitors, PIs not only inhibit the production of viruses from newly formed cells, but also act upon chronically infected cells. Notable virological, immunological and clinical benefits have been observed when PIs are used as part of a triple combination therapy. For therapy to be deemed beneficial, there must be an initial and sustained drop of at least 0.51 μg of HIV RNA from pre-treatment levels, an increase in CD4+ cells, and the postponement of disease progression.

Monotherapy, may lead to the emergence of variant strains of HIV, subsequently leading to clinical failure. Combination therapy (Table 2) renders an additive and synergistic effect, and precludes the patient from developing immediate resistance, as the rate of replication and thus mutation decreases. Not only is irreversible damage to the patient’s immune system prevented, but also effective reductions in viral load and suppression of HIV replication result. This is the optimal mainstay for antiretroviral therapy.

**Table 2: Possible Combination Therapies used in HIV Infection**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Two Nucleoside analogues + one protease inhibitor</td>
</tr>
<tr>
<td>ZDV + ddl/ddC/3TC + IND/NEL/RIT</td>
<td></td>
</tr>
<tr>
<td>d4T + ddl/ddC/3TC + IND/NEL/RIT</td>
<td></td>
</tr>
<tr>
<td>2)</td>
<td>Two nucleoside analogues + Non nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>ZDV + ddl/ddC/3TC + NVP/DEL</td>
<td></td>
</tr>
<tr>
<td>d4T + ddl/ddC/3TC + NVP/DEL</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Barry et al, British Journal of Clinical Pharmacology 1998; 45: 221-228.

**RESISTANCE TO PROTEASE INHIBITORS**

Resistance to PIs has been observed, but resistance to a given PI may not confer resistance to another PI. Plasma viral RNA levels are assessed to determine if resistance has developed. Thus, measurements of HIV RNA will allow the clinician to continue or to modify a therapeutic regimen. A detectable level of virus does not necessarily constitute treatment failure. The degree of therapeutic success or failure depends on the relative changes compared to baseline. Treatment should continue as long as the patient is benefiting and should change if the patient’s viral load rebounds towards baseline.

Dosing is an important consideration in PI therapy. Sub-optimal doses, or non-compliance to a dosing regime, will trigger resistant mutants. Maintaining high plasma trough concentrations by increasing the dosage will delay the onset of mutations. This is in contrast to NRTIs where increasing the dose will cause an increase in toxicity. To avoid possible resistance, drug monitoring of PI levels is necessary to keep their levels at supra-threshold doses for maximal antiviral effects. There is great subject variability in the concentration of plasma PI levels due to differences in each patient’s Cytochrome P450 CYP3A4, the route of PI metabolism. As such, dosage must be tailored to allow for maximum efficacy and minimum toxicity, otherwise it is ineffective if the concentration is sub-optimal. Patient compliance to combination therapy is of utmost importance to strengthen the efficacy of these drugs, and monitoring may determine whether the patient is compliant.

The current recommendations of initiating therapy include any HIV seropositive symptomatic patient, and asymptomatic patients who present with an elevated viral load of greater than 5000 HIV-1 RNA copies/mL or decreased CD4+ counts of less than 300μL. Triple combination therapy should be commenced immediately, with the aim of reducing the viral load and increasing the CD4+ counts. Laboratory measurements of viral RNA and CD4 counts, and clinical symptoms should be accounted for every three months to ensure that viral suppression is maintained and clinical progression
of HIV is attenuated. The most significant factor that determines whether therapy should be revised is the viral load. Monitoring of HIV RNA levels allows for such a decision to take place. Toxicity or drug intolerance may also compel the physician to alter therapy. Substitution of one drug is not indicated in therapeutic failure, as resistance is most likely to develop. In effect, an entirely different combination (Table 3) should be administered so as to prevent overlapping toxicity and cross-resistance. The newly administered drugs should also not have been previously given to the patient. The effectiveness of this new regime can be evaluated as before. It is not recommended to cease antiviral therapy at any stage of HIV infection, as viral load and resistance may develop. Therapy is deemed beneficial throughout the progression of HIV.

**Pharmacokinetic Profiling of Protease Inhibitors**

The focus of this discussion will concentrate on the two most frequently administrated PIs, saquinavir and ritonavir. There is a complexity of pharmacokinetic parameters (Table 4) that may influence their antiviral effects, these include bioavailability, protein binding, metabolism, toxicity and drug interactions.

PIs are characterized by their varying bioavailability due to their absorption from the gastrointestinal tract and first pass metabolism by CYP3A4. P-Glycoprotein may also contribute to the bioavailability of these agents. Saquinavir, in its hard-gel capsule, has significant antiviral effects, although its oral bioavailability is approximately 4-5%. However, a soft-gel capsule has been developed to increase the Area Under Curve (AUC) and thus the bioavailability of the drug, which consequently will allow for higher plasma drug levels, more potent antiviral effects, and perhaps a reduction in the dosage administered at any one time.

Ritonavir is characterized by higher bioavailability. High-fat meals and an increase in gastric pH will increase the bioavailability of both ritonavir and saquinavir by facilitating absorption of these basic drugs. These factors may affect oral bioavailability of PIs. A goal in devising new therapeutic agents is to overcome these barriers and promote maximum bioavailability and antiviral activity.

Antiviral efficacy is affected by the high affinity of PI to plasma proteins. a,-acid glycoprotein binds to both saquinavir and ritonavir resulting in low unbound plasma concentration of the drugs. A low free-drug concentration reduces the antiviral activity. However, therapeutic efficacy should be noted despite the extent of protein binding of PIs, if the unbound concentration exceeds the inhibitory concentration to inhibit the replication of the virus by 90% (IC50).

A low free-drug concentration reduces the antiviral activity. However, therapeutic efficacy should be noted despite the extent of protein binding of PIs, if the unbound concentration exceeds the inhibitory concentration to inhibit the replication of the virus by 90% (IC50). A side effect of PI therapy is evident. There is slight toxicity associated with the use of these drugs, although they are, in general, well tolerated. Of note, all four currently licensed PIs cause hyperglycaemia and a redistribution of fat, and must be used in caution in pregnancy, hepatic impairment and in hemophilia.

Saquinavir is associated with gastrointestinal

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**Table 3: Initial and Alternate Antiretroviral Regimens**

<table>
<thead>
<tr>
<th>Initial Regimen</th>
<th>Alternate Regimen</th>
</tr>
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<tbody>
<tr>
<td>ZDV + ddi + IND</td>
<td>d4T + 3TC + NEL</td>
</tr>
<tr>
<td></td>
<td>d4T + 3TC + NVP</td>
</tr>
<tr>
<td></td>
<td>RIT + SQV + 3TC</td>
</tr>
<tr>
<td>ZDV + 3TC + IND</td>
<td>d4T + ddi + NEL</td>
</tr>
<tr>
<td></td>
<td>d4T + ddi + NVP</td>
</tr>
<tr>
<td></td>
<td>RIT + SQV + d4T</td>
</tr>
<tr>
<td>d4T + 3TC + IND</td>
<td>ZDV + ddi + NEL</td>
</tr>
<tr>
<td></td>
<td>ZDV + ddi + NVP</td>
</tr>
<tr>
<td></td>
<td>RIT + SQV + ZDV</td>
</tr>
<tr>
<td>ZDV + ddi + NVP</td>
<td>d4T + 3TC + IND</td>
</tr>
<tr>
<td></td>
<td>ZDV + 3TC + IND</td>
</tr>
</tbody>
</table>

*Adapted from Barry et al, British Journal of Clinical Pharmacology, 1998, 45: 221-228.*

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**Table 4: Pharmacokinetic Profile of Saquinavir, Ritonavir and Indinavir**

<table>
<thead>
<tr>
<th>Kinetic Parameter</th>
<th>Ritonavir 300mg, 12 hourly</th>
<th>Saquinavir 600 mg, 8 hourly</th>
<th>Indinavir 800 mg, 8 hourly</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (mg/L)</td>
<td>3-8</td>
<td>0.04-0.10</td>
<td>5-11</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>1-3</td>
<td>1-2</td>
<td>1-2</td>
</tr>
<tr>
<td>AUC (mg*h/L)</td>
<td>5-45</td>
<td>0.01-0.04</td>
<td>14-28</td>
</tr>
<tr>
<td>$t/z_{1/2}$ (h)</td>
<td>3</td>
<td>7-12</td>
<td>2</td>
</tr>
<tr>
<td>$CL/F$ (L/h)</td>
<td>6-14</td>
<td>50-100</td>
<td>50-80</td>
</tr>
<tr>
<td>Plasma Protein Binding (%)</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td>60</td>
</tr>
<tr>
<td>Renal Elimination (%)</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>10-15</td>
</tr>
</tbody>
</table>

*Abbreviations: AUC=area under the concentration-time curve; CL/F=clearance adjusted for bioavailability; $C_{\text{max}}$ = maximum plasma drug concentration; $t_{\text{max}}$ = time to maximum plasma drug concentration; $t/z_{1/2}$ = elimination half-life associated with the slope of the concentration time curve.*

*Adapted from Barry et al, Clinical Pharmacokinetics 1997 March; 32 (3): 194-209.*
disturbances, although they are dose-related. Liver enzyme markers, transaminases and creatine phosphokinase, are also somewhat elevated. Patients may also complain of headaches, mucosal ulceration, rashes, paraesthesia, asthenia and peripheral neuropathy.

Ritonavir’s adverse effects are usually observed in the initial stages of therapy which then subside. Similar to saquinavir, ritonavir may elicit gastrointestinal disturbances. In a select few number of patients, circumoral and peripheral paraesthesias, weight loss, and altered taste sensation have been reported. Although not clinically significant, there are elevations of aminotransferases and serum triglyceride levels. Pancreatitis has been reported in some patients, and necessitates withdrawal of the drug. In addition, thrombocytopenia and other haematological abnormalities may occur.

**Drug Interactions: General Principles for Protease Inhibitors**

The CYP3A4 isoform is responsible for the metabolism of PIs with slight activity from CYP2D6. It is also responsible for the metabolism of a wide spectrum of drugs. As a result, significant drug-drug interactions either by induction and inhibition are likely to occur. Further drug interactions may be the consequence of modified drug absorption, altered ability of renal excretion or drug displacement from binding sites on plasma proteins.

PIs are all inhibitors of this system, albeit to varying degrees. Ritonavir is the most potent CYP3A4 enzyme inhibitor, whereas saquinavir has been determined to be the weakest. An inhibitor will cause the AUC of its co-administered drug to increase, thereby, increasing the drug efficacy, response, and potential toxicity. These effects are exaggerated in drugs with a narrow therapeutic margin such as warfarin and theophylline. An inducer will result in lower plasma levels. This is problematic in HIV therapy, as sub-therapeutic drug concentration levels may result in decreased drug efficacy, and exacerbate patient adherence to the dosing regimen, whereby long-term compliance, maintained virological, immunological and clinical effects.

Ritonavir is a potent inhibitor and inducer. Upon initial administration of this drug, higher than expected plasma levels of the drug result. However, steady state is reached following a two-week period, as autoinduction of the CYP3A4 subsequently causes the concentration to return to baseline levels. AUC and C_{max} values tend to decrease after multiple dosing, which confirms the induction of its metabolism. This may account for the increased toxicity observed upon initiating ritonavir therapy.

These pharmacokinetic parameters of ritonavir may prove to be favourable in co-administration with other PIs. Since ritonavir is the most potent inhibitor of this class, the bioavailability of other drugs is subsequently increased, with a decrease in their metabolism and increases in their trough concentrations. Ritonavir pharmacokinetics are barely affected. Saquinavir’s bioavailability increases remarkably, as ritonavir inhibits the former’s first pass metabolism. Virological, immunological and clinical improvements have been noted with amelioration of the clinical condition with concurrent administration. This pharmacokinetic interaction may allow for a reduction in the dosage of the PI to reduce the toxic side effects that may affect each individual patient. Concurrent administration of two PIs has allowed administration of the drugs from three times daily, to twice daily as the plasma levels of saquinavir are increased more than ten-fold. This has important consequences on patient adherence to the dosing regime, whereby long-term compliance, maintained and increased antiviral effects, and minimal drug-resistant mutations will arise. This also is important, pharmacoeconomically, as striking reductions in costs may follow.

**Drug Interactions: Non-Anti-HIV Agents**

HIV seropositive patients, and especially those who have progressed to the AIDS stage by
acquiring an opportunistic infection, are most likely recipients of other medication, such as rifampicin and ketoconazole, and these concurrently administered drugs may be involved in drug-drug interactions. The caregiver must be conscious of the potential interactions between these therapeutic agents, as the efficacy of either the PI or the agent administered may be compromised. For instance, simultaneous administration of ritonavir and rifampicin, a drug used in pulmonary tuberculosis, will decrease the AUC of the former by 35%. As such, to maintain a therapeutic dose of this anti-HIV drug, without causing any undue toxicity, the dosage of the PI, depending on the patient, should be somewhat increased. Careful monitoring of plasma levels is required. The antifungal agent ketoconazole, will increase the AUC of saquinavir, causing this interaction to be extremely low, as the oral bioavailability of the PI reaching the circulation is low.

Other interactions of note include the co-administration of anti-histamines, such as terfenadine and astemizole, as potentiation of the QT interval may arise. Drugs metabolised by the CYP3A4, CYP2D6, CYP2C9, phenobarbitol, and other CYP3A4 medications. Drugs such as benzodiazepines, cisapride, phenytoin and astemizole, as potentiation of the QT interval may arise. Administration of anti-histamines, such as terfenadine and astemizole, as potentiation of the QT interval may arise. Reaching the circulation is low.

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Ritonavir may further interact with benzodiazipines, cisapride, phenytoin and phenobarbitol, and other CYP3A4 medications. Drugs metabolised by the CYP3A4, CYP2D6, CYP2C9, CYP3A, and CYP1A2 will interact with Saquinavir. Clinicians must be fully aware of the possible drug interactions that involve HIV protease inhibitors and avoid or adjust medication which may alter the drug’s efficacy, as there is an immense possibility that drug interactions involving PIs may occur.

**Conclusion**

The emergence of protease inhibitors has revolutionized the therapy for HIV disease as they have been shown to increase life expectancy in these patients. However, the long term effects of PIs are not yet known, and may prove cumbersome in future treatment schedules. However, the drug’s effectiveness and the patient’s clinical outcome are dependent on the clinician’s ability to properly administer the drugs, and the patient’s compliance to the therapeutic regime. Optimal dosing of supra-threshold levels is required to maintain antiviral efficacy and to prevent the appearance of resistant strains. Therapeutic drug monitoring of HIV RNA, CD4+ counts and PI levels appear to assist in the management of HIV therapy and is clearly indicated to provide a desirable outcome. The improved understanding of the molecular basis of the disease will lead to the development of therapy that can target the entry and integration of HIV into cells, as well as possibly focusing on the introduction of HIV vaccines. These new approaches, in conjunction with the existing treatment, should drastically alter the current management and prognosis of this disease.

**References**

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