The role of therapeutic drug monitoring in treatment of HIV infection

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Introduction

Looking back over the development of antiretroviral therapies (ART) for the treatment of HIV infection, probably the turning point in generating a belief that there were real grounds for optimism of 'therapeutic success' came with the introduction of protease inhibitors (PIs) in 1995. As a component of antiretroviral therapy, PIs produced a dramatic decrease in mortality and morbidity in HIV infection [1] most clearly demonstrated by the reduction of opportunistic infections and hospital admissions. Today, a triple drug combination regimen containing nucleoside reverse transcriptase inhibitors (NRTIs) plus PIs or non-nucleoside reverse transcriptase inhibitors (NNRTIs) constitutes the standard of care for patients commencing therapy (see Table 1).

Despite its success, combination ART still lacks sufficient potency and durability. Large prospective studies [2] suggest that up to 50% of HIV-positive patients will fail to achieve adequate suppression of plasma HIV RNA (i.e. <50 copies/ml) with any of the current ART regimens. Even if this is achieved, viral rebound develops in a significant proportion of patients within 1 year of follow-up [3–5]. For example, in the Swiss cohort study [3], rebound HIV viraemia (from <400 copies/ml to detectable) was approximately 10% per year in ART-naïve patients commencing therapy and 20% in ART-experienced patients switching therapy. Although 80% of ART-naïve patients achieved viral load below 400 copies/ml at 6 months, this was only sustained in 66% at 30 months and treatment changes were necessary in approximately half of patients by 24 months. In the Frankfurt cohort [4], over half of patients developed viral rebound within 12 months of achieving undetectable RNA. There are also a growing number of patients who

fail treatment despite exposure to most antiretroviral agents and consequently receive salvage therapy, which may include 'mega-ART' regimens. Such regimens are associated with increased potential for toxicity and serious drug interactions. In this context, some of the most pressing clinical pharmacology questions are:

- Why do some patients fail treatment regardless of the regimen selected?
- How can existing therapies be improved or optimized?
- Will the new drugs in development show significant advantage?

Treatment failure is clearly multifactorial, and may include the development of antiviral resistance, poor adherence to therapy and pharmacokinetic reasons. This review will focus on pharmacokinetic variability as an important consideration in treatment failure and current approaches to address the problem.

Pharmacokinetic variability is particularly important in relation to PIs – a group of peptidomimetic drugs with considerable inter and intra-individual variability in plasma levels and marked potential for drug interactions leading to reduced or elevated PI plasma concentrations [6, 7]. Reduced concentrations will potentially compromise efficacy while elevated concentrations will predispose to adverse events. When considering the role of therapeutic drug monitoring (TDM) in antiretroviral therapy, the primary focus is therefore on PIs. However before proceeding to develop the arguments for TDM of PIs it is important to highlight the main issues around plasma concentrations of the other two main classes of antiretrovirals.

For NRTIs, establishing a relationship between plasma concentration and antiviral effect has been difficult simply because it is the intracellular triphosphate anabolite that is the active moiety (Figure 1). Plasma concentrations of parent nucleosides and intracellular concentrations of triphosphates show only a weak correlation. Figure 2

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displays data from studies with zidovudine (ZDV) and lamivudine (3TC) [8, 9]. Therefore meaningful data would require cell separation (i.e. to generate peripheral blood mononuclear cells, PBMCs), a technique which is time consuming, followed by analysis of the active triphosphate, a procedure that is currently available only in a handful of laboratories. This effectively precludes the routine use of TDM for NRTIs in clinical practice until such time as a more rapid throughput analytical system is developed.

Data from pharmacokinetic studies of NNRTIs indicate that two of the drugs, efavirenz and nevirapine, have a prolonged half-life, normally achieve adequate steady-state plasma concentrations during a dosing interval and have pharmacokinetics which are less variable than those of PIs. Although we would not entirely rule out a role for

TDM of NNRTIs in some circumstances (e.g. in relation to CNS side-effects of efavirenz), presently attention is focused on PIs.

Table 1 Currently licensed antiretrovirals.

Protease inhibitors (PIs)	Nucleoside reverse transcriptase inhibitors (NRTIs)	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
Amprenavir	Abacavir (ABC)	Delavirdine
Indinavir	Didanosine (ddI)	Efavirenz
Nelfinavir	Lamivudine (3TC)	Nevirapine
Ritonavir	Stavudine (d4T)	
Saquinavir	Zalcitabine (ddC)	
(soft gel, hard gel)	Zidovudine (ZDV)	
Recommendation for initial treatment is:		
PI* + 2 NRTIs		
NNRTI + 2 NRTIs		
3 NRTIs†		

Note: *PI might be a combination of two PIs. †Not in official guidelines, but increasingly used.

Figure 2 The relationship between intracellular nucleoside triphosphate and plasma concentration of parent drug for (a) zidovudine (ZDV) and (b) lamivudine (3TC) (○ 150 mg, ● 300 mg). Note the weak correlation between area under the curve in cells and plasma for each drug.

Figure 1 Activation pathways of nucleoside analogues.

The argument for using TDM for PIs

(i) Drug concentrations correlate with antiviral effect

This probably represents the strongest case for TDM. An association between plasma saquinavir levels and virological response was observed in patients participating in a dose ranging study of the original hard-gel formulation [10] and upon initiation of ART [11]. Gieschke *et al.* [12] investigated the relationship between systemic exposure to monotherapy saquinavir (soft-gel formulation administered at 400, 800, 1200 mg three times daily) and plasma HIV RNA and CD4 cell counts using empirical mathematical modelling and indicated the area under the plasma concentration–time curve which gives maximal viral suppression (Figure 3). In the ADAM study, plasma saquinavir and nelfinavir concentrations were strongly associated with the initial rate of HIV clearance [11].

Dose ranging monotherapy studies of zidovudine and didanosine have also demonstrated a relationship between dose and clinical response. There was rapid emergence of antiviral resistance with the use of lower than recommended doses [13–16].

A phase I/II study of zidovudine demonstrated a good relationship between zidovudine exposure (AUC and C_{min} levels) and virological response [17]. The TRILEGE study [18] reported an association between low zidovudine levels and treatment failure, although, a similar US study (ACTG 343) failed to observe this association [19]. Several other US [20, 21] and European [22, 23] studies have reported an association between zidovudine levels (using AUC, C_{max} , C_{min} or concentration ratios) and virological response in both adults and children. The evidence therefore is that maintaining therapeutic levels is potentially critical for PIs, not just in preventing drug resistance, but also

Figure 3 Dose ranging study (NV 15107) to determine the optimal dose of saquinavir soft-gel (Fortovase). (▲ Fortovase 400 mg three times daily, ■ Fortovase 800 mg three times daily, ● Fortovase 1200 mg three times daily. From Gieschke *et al.* [12] with permission of Adis Press.

cross-resistance with other PIs. Exposure of a patient to subtherapeutic levels of a PI may result in the stepwise accumulation of mutations in the HIV protease with subsequent resistance to both the prescribed drug and other class members. Recently, results from the VIR-ADAPT study have been reported [24]. This is a randomized controlled trial of HIV genotyping to guide decision making *vs* standard of care (SOC). However, plasma samples were also analysed retrospectively for PI concentrations and patients were categorized as having optimal or suboptimal concentrations based on trough concentrations being above or below the IC_{50} of the drug. The results provided evidence of the benefit of both genotyping and having optimal PI concentrations. For example, at 6 months there was only a -0.23 log drop in viral load in patients receiving SOC with suboptimal drug concentrations, but a -1.3 log drop in patients having both genotyping and optimal drug concentrations.

(ii) Variability in plasma drug concentration.

PI concentrations in plasma show marked interpatient variability following standard dosing regimens. Based on studies with hard-gel saquinavir showing a greater than 20-fold variability in trough concentrations we suggested that TDM of saquinavir could have a role in improving therapeutic outcome [25]. Our conviction has only increased as we have developed a TDM service for selected patients in the UK. Figure 4 shows trough PI concentrations from this study. There are a number of features. Clearly there is huge interpatient variability for all the PIs. Note, for example, that a high proportion of patients receiving the original hard-gel formulation of saquinavir have trough concentrations (C_{trough}) below the target minimum effective concentration (MEC). The latter is derived substantially from *in vitro* studies where the concentration of each PI to inhibit wild-type HIV is determined (IC_{50} or IC_{95} values) in the presence of 50% human serum [26]. The latter is important since PIs (particularly saquinavir, nelfinavir, zidovudine, amprenavir) are extensively bound to plasma proteins. Using these protein-corrected inhibitory concentration values we and others have estimated the *in vivo* MEC for each PI (Table 2).

Plasma concentrations following the introduction of soft-gel saquinavir are generally much higher, although some patients still present with trough concentrations below the desired value. Zidovudine, zalcitabine and didanosine show similar variability. It should be stressed that the concentrations presented in Figure 4 are from both daily clinical practice and some carefully monitored clinical trials. They represent a cross-section of patients, some of whom will have undergone TDM because of suspected virological failure, drug interaction or a change in therapy.

Figure 4 Trough plasma concentrations of saquinavir, indinavir and nelfinavir after various dosing regimens alone and in the presence of ritonavir. Data from Liverpool HIV Pharmacology Group. hcg = hard-gel capsule; sgc = soft-gel capsule. The horizontal line indicates the minimum effective concentrations.

Table 2 Range of target minimum effective concentrations (MEC) based on protein binding corrected IC_{95} values for individual PIs.

Protease inhibitors (PIs)	MEC	
	<i>nM</i>	<i>ng ml⁻¹</i>
Amprenavir	588–785	300*–400
Indinavir	97–160	60–100*
Nelfinavir	700–1228	400*–700
Ritonavir	2080–2910	1500–2100*
Saquinavir	150–300	100*–200

Note: different laboratories have used slightly different cut off values.

*Indicates values used in the Liverpool HIV Pharmacology Group.

In relation to the marked interpatient variability, although it is difficult to pinpoint a single factor, we know that there are clear effects of food (on absorption), drug interactions and hepatic dysfunction. Since PIs are substrates for enterocytic and hepatic CYP3A4 [6] and P-glycoprotein (P-gp) [27–32], drugs which inhibit CYP3A4 (e.g. other PIs, azole antifungals, macrolide antibiotics) or P-gp may result in marked elevation of PI concentrations. Conversely, the induction of CYP3A4 by drugs such as some NNRTIs, rifampicin and rifabutin may decrease PI concentrations. A patient failing on one regimen will be given at least two new drugs and in salvage therapy increasingly complex combinations of drugs (as many as 4–8 agents) which may include PI–PI combinations plus an NNRTI. These regimens have considerable potential for complex drug interactions and toxicity. For a complete listing of the important drug interactions in HIV therapy see <http://www.hiv-druginteractions.org>.

(iii) Drug concentrations may correlate with excessive toxicity

Reversible liver toxicity is seen with high-doses of PIs or dual PI combinations. High peak plasma concentrations (C_{max}) of indinavir are associated with urological complications [33]. Similarly, high C_{max} values of ritonavir are related to circumoral paraesthesia [34]. We have successfully changed ritonavir dosing regimens (e.g. to 300 mg four times daily) to overcome this effect [34]. Data are emerging which suggest an association between saquinavir and indinavir levels and plasma triglycerides [35].

(iv) Altered clearance in hepatic dysfunction

Pre-existing liver impairment, particularly with coexistent chronic hepatitis B or C infection is not uncommon in HIV infection. Wide variability in the pharmacokinetics of nelfinavir in a small group of patients has been demonstrated [36] and liver dysfunction is likely to affect the disposition of all PIs. In the ANRS EP11 Study of over 1200 patients receiving PIs, most cases of serious liver toxicity (>grade 3) were seen in patients co-infected with hepatitis C virus [37]. Plasma levels may assist in optimizing doses, minimizing hepatotoxicity and discriminating between drug toxicity and other causes of liver impairment.

(v) To assess adherence to therapy

Lack of adherence to therapy due to the complexity of drug regimens is a major problem. HIV treatment appears to be very 'unforgiving' in this respect, since therapeutic failure is very closely associated with failure to adhere to

prescribed therapy. For example, a study using pill bottles fitted with electronic caps demonstrated that successful HIV suppression in patients with >95% adherence was 81%, with 90–95% adherence this was 64%, with 80–90% adherence this was 50%, with 70–80% adherence this was 25% and with <70% adherence only 6% patients achieved successful HIV suppression [38]. Although the plasma half-life of PIs is comparatively short (2–8 h), monitoring of plasma drug levels may be useful for selected patients in whom nonadherence is suspected [39]. In this circumstance, TDM may identify poor adherence although adequate plasma drug levels would not automatically imply good adherence.

Methods of analysis

Protease inhibitors are assayed using high performance liquid chromatography (h.p.l.c.) or liquid chromatography–mass spectrometry (LC–MS). The limits of quantification are around 25–50 ng ml⁻¹ which are low enough to monitor trough concentrations of individual PIs. Quality assurance programmes are only just beginning to emerge. In Europe, the main QA programme is organized by the University Medical Centre, St Radboud, the Netherlands with 14 laboratories involved as of August 2000.

Potential problems with TDM

One of the major problems with TDM is knowing the target concentration. Minimum target PI concentrations

have largely been defined on the basis of monotherapy concentration–effect modelling or *in vitro* (IC₉₅) data for laboratory or clinical isolates of HIV, with allowance made for protein binding. But how are these values affected by other antiretroviral agents in a given combination? Are data derived from studies using PI monotherapy or complex four drug regimens including dual PIs, appropriate to the general clinic population of HIV-positive patients?

In using a single target trough concentration, we assume that all patients have viral isolates with the same susceptibility. In reality many patients with resistant isolates require higher concentrations. Interindividual variability in drug absorption (for example with diet or according to disease stage) or changing patterns of adherence may confuse the picture and give TDM a poorer predictive value.

Finally there is little agreement concerning the best measure to use: AUC, $C_{\min}$ (C_{trough}), or concentrations ratios (i.e. concentration obtained at any time point related to population profile data). While AUCs represent a robust measure, there are logistical difficulties in instituting their use on a wide scale. There is currently a move to consider using $C_{\min}/IC_{50}$ values (IC_{50} being for the patient isolate) and this would certainly seem to be the way forward. In this connection the term inhibitory quotient (IQ) has been introduced where $IQ = C_{\min}/IC_{50}$ and there is clearly an advantage if the IQ for a PI is in excess of 1. Figure 5 illustrates the deriving of IQ values.

- ◆ A parameter to estimate *in vivo* potency of antiretroviral drugs.
- ◆ $C_{\min}/IC_{50}$ is suitable for across-study, across-patient and across-drug comparison
- ◆ $IC_{\min}$ is generated from *in vivo* pharmacokinetic data (see upper graph)
- ◆ IC_{50} or IC_{95} are generated *in vitro*, increasing drug concentration until 50% or 95% of the virus is inhibited
 - > how close the IC_{50} and IC_{95} values are to each other depends on how steep the curve is (see lower graph)
 - > how reliable the values are depends on the system used to measure them
- ◆ IQ (inhibitory quotient):
 - > $IQ = \text{trough concentration in plasma} / \text{concentration required for inhibition in vitro}$
 - > gives an index of how far the concentration of a drug *in vivo* is in excess of the viral IC_{50}

Figure 5 Inhibitory quotient.

Clearly the 'want' for TDM is widespread while the 'need' has yet to be fully established. Questions such as 'Does TDM improve patient outcome?' and 'Is it cost-effective?' urgently need to be addressed. Assessing the costs of PIs and of monitoring their levels is a health service issue of great immediacy. It is clear that randomized controlled trials to assess TDM are urgently required. If such studies are not performed there will be mounting demand for TDM to be instituted, a circumstance analogous to viral resistance testing. Results of one TDM study, the ATHENA trial in the Netherlands, should be reported in late 2000 [40]. In 1999, the Pharmacology Committee of the US AIDS Clinical Trials Group (ACTG) in a position paper [41] issued guidelines stating that routine TDM was not recommended 'except in the context of supervised clinical studies designed to assess the utility of TDM'. This cautious approach needs to be viewed in the context of increasing numbers of patients failing therapy because the durability of ART appears to be limited. While newer PIs, NNRTIs and NRTIs are being evaluated, there are no imminent plans for the introduction of a new major class of compound into Phase III studies. The urgent need to improve efficacy and preserve treatment options has therefore led to calls for the institution of routine TDM by many clinicians, leading experts and patient advocacy groups particularly in Europe. Despite the lack of definitive studies to evaluate the clinical benefits of and indications for TDM, some national treatment guidelines (e.g. BHIVA [42]) have incorporated TDM as an option for the management of HIV infection.

Pharmacoenhancement

A critical examination of current PIs suggests that there is a need for better drugs that achieve higher plasma concentrations reliably without associated toxicity. This urgent need to improve efficacy and preserve treatment options has led to the use of ritonavir as a pharmacoenhancer with the aim of obtaining plasma concentrations of unbound drug that are in excess of the IC_{95} of both WT and mutant virus [43, 44]. The benefits include improving bioavailability through inhibition of first-pass loss (e.g. metabolism by CYP3A4 and efflux by the transporter P-gp) and reducing clearance. Data from twice daily dosing are convincing (e.g. indinavir-ritonavir 800/100 mg or 400/400 mg (Figure 6), lopinavir-ritonavir 400/100 mg, saquinavir-ritonavir 400/400 mg, amprenavir-ritonavir 600/100 mg) although it is probably too early to say if the data from once daily dosing are anything more than promising. Eventually we will move into the once daily single agent PI (without pharmacoenhancement) and there are drugs in development. A further consideration is whether the pharmacoenhancement of ritonavir will

Figure 6 Effect of adding ritonavir to indinavir (taken from [45]). The figure shows plasma concentration-time curves for indinavir when administered alone (800 mg, 8 hourly, fasting; IND alone) and with ritonavir (400 mg, 12 hourly with ritonavir 400 mg and food; IND + RIT). The dotted line represents the IC_{50} of indinavir for WT HIV.

overcome any enzyme induction effect of co-administered NNRTI. Although this appears to hold for saquinavir/ritonavir + efavirenz, amprenavir/ritonavir + efavirenz, lopinavir-ritonavir + nevirapine, recent data indicate this is not so for lopinavir-ritonavir + efavirenz.

One issue with pharmacoenhancement is whether an increase in toxicity will be seen. Here TDM may have a role in dose reduction.

Conclusion

In conclusion, there are strong pointers to an important role for TDM in ART and in particular if TDM is shown to be beneficial in controlled clinical trials, it will assist clinicians in optimizing the management of HIV-positive patients and by delaying the onset of virological failure, preserve future treatment options. We also need to remember that expert interpretation of a concentration is absolutely critical to understanding what the results actually mean. This is not therapeutic drug **measurement** but therapeutic drug **monitoring**.

Note: A number of cited references are from recent international meetings. If any reader has difficulty locating any of these references please contact the authors.

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